MAKING LIVES EASIER FOR ANIMALS IN RESEARCH LABS

Discussions by the Laboratory Animal Refinement & Enrichment Forum

EDITED BY VERA BAUMANS, CASEY COKE, JENNIFER GREEN, ERIK MOREAU, DAVID MORTON, EMILY PATTERSON-KANE, ANNIE REINHARDT, VIKTOR REINHARDT AND PASCALLE VAN LOO

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1. Introduction and Acknowledgements

This book is a collection of electronic discussions that took place on the Animal Welfare Institute's Laboratory Animal Refinement & Enrichment Forum (LAREF) between October 2002 and May 2007. The forum serves the international animal care and animal research community to promote animal welfare and improve scientific methodology by avoiding or minimizing stress and distress resulting from husbandry and handling practices.

Of more than 5,000 comments posted, approximately 3,000 were selected for this book because they have practical animal welfare relevance and are based on first-hand experiences about ways to improve the conditions under which animals are housed and handled in research facilities.

I am grateful to the 62 animal technicians, 38 researchers, 13 veterinarians, two cage designers, two editors, and two librarians who contributed these comments:

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Each comment was edited without changing its content. Different comments with equivalent content were summarized in one comment. References from the published literature were added during the editing process to further contribute to particular discussion topics. The questions and a summarizing conclusion of each discussion are printed in bold.

It is my wish that this book will help to make life easier for animals in research laboratories, thereby improving the scientific quality of research data collected from them. May these discussions inspire and encourage all those who are responsible for the care and well-being of animals in research labs to express their compassion in action. The way we treat animals predetermines our own emotional well-being.

Mt. Shasta, California
May, 2007

Viktor Reinhardt
Moderator of LAREF

2. Basic Issues

2.1. How to Refer to an Animal—Using the Proper Pronoun

It is a custom in biomedical research to use the pronoun "it" rather than "he" or "she" when referring to an animal, even if the animal is assigned to a project in which gender-related phenomena—e.g., reproductive physiology/behavior—are studied. I want to question whether it is really appropriate to use the pronoun "it" for an intact animal.

I once referred to individual study animals as he/she. The principal investigator asked me to use "the animal" instead and lectured me that it is not scientifically appropriate to personalize an animal.

Perhaps you do "personalize" an animal, but this does not change the fact that using the gender-appropriate pronoun "he" or "she" is more accurate than using the pronoun "it," as if the subject had no gender. Why would it not be scientifically appropriate to refer to intact animals with the proper pronouns "he" or "she?" I have always called animals, whether research subjects or not, he or she. To refer to an animal as "it" is to remove oneself from a living creature and regard and treat this animal like a thing. I think animals deserve some respect, and calling them "he" or "she" is the least we can do. "Personalizing" the animals provides them basic assurance that you are considerate of the fact that they are living creatures who do feel pain, discomfort and distress in a similar manner as you do, and that their well-being is impaired when you expose them to discomfort, pain and distress. You will probably do your best to promote their well-being, which will also benefit scientific methodology. Not referring to an intact animal as "he" or "she" but as "it" is scientifically less appropriate than the reverse. After all, a "female" is not a neuter, and a "male" is also not a neuter. No scientist can, for example, study reproductive phenomena in an animal who is neither a "she/female" nor a "he/male." Why pretend that animals have no reproductive organs and label them with the pronoun that we use for dead things, i.e., objects? We usually treat "things" differently than animals, because we know that they are not sentient, and hence do not suffer. Once we label an animal as a thing, the risk arises that we will treat the subject accordingly, for example, as a "standardized biological research tool" (Hummer, 1965) and no longer as a living creature.

The animals who serve us for experimental purposes should be treated with respect. They do deserve to be seen and treated accordingly as sentient beings who are, at the very least, referred to by their biological gender. At our facility we try to use the correct pronouns "he" or "she" for all our animals. Perhaps not surprisingly, the worst offenders for labeling an animal "it" are our surgeons! We do discourage our personnel from using the pronoun "it," since we do not want to encourage them to regard animals as mobile test tubes.

I am not convinced that using the correct pronouns "he" and "she" will change the attitude of people who regard animals as sophisticated versions of "test tubes." As a clinical veterinarian, I suspect that animals assigned to biomedical research have traditionally been labeled as quasi-objects in an attempt to protect the researcher from ethical concerns about the fact that he or she inflicts pain, distress, and probably also suffering on conscious creatures. The way we refer to animals in our language does reflect our attitude toward them, and the way we attend to their basic needs for well-being and safety. I guess the research laboratory is a place in which this kind of respect for life is not in high regard, because the research itself often implies the mutilation and killing of animals who are, after all, living beings just like scientists themselves.

As caregivers, we do not use the pronoun "it" when referring to an animal. An animal is not an object! We do not think that calling an animal "he" or "she" encourages anthropomorphism, but that it does acknowledge the fact that we are dealing with an individual sentient being who can feel discomfort, pain and distress in very similar ways as we do. Calling individual animals "he" or "she" helps us deal with something that deep down, we are not really comfortable with—namely the fact that these animals have no choice about deciding whether they want to be used in research and then killed.

Referring to an animal as "it" is neither correct nor scientific, because it overlooks the fact that animals, just like humans, have a biological gender. Therefore, they should be referred to accordingly with the correct pronouns "he" or "she." When we label an animal with the incorrect pronoun "it," we risk treating the animal like an inanimate object incapable of feeling discomfort, pain and distress.

2.2. Higher- Versus Lower-Order Species

It seems that "refinement" in the use of animals for research includes choosing lower-order species rather than higher-order species, presumably due to the assumption that the lower-order animals suffer less and that their use in experiments poses fewer ethical problems. Where do we draw the line?

To me, nonhuman primates seem to be sufficiently different from other mammals—in having a sense of self and of the future—to deserve particular

consideration, but what about dogs versus mice? There is a great cultural difference, in that humans tend to view dogs as beloved pets, and mice as abominable pests. But does that mean that the mouse is of a lower order, and therefore suffers less from research than a dog? As scientists, using animals for "our" research, we should be in the position to go beyond this weird idea of animals being of a lower or higher order. We are at a great risk of not treating our research subjects very well when we consider them of "lower" order, and by doing so, jeopardize the quality of our research methodology. When colleagues tell me that mice are lower mammals who cannot suffer from anything akin to human mental disorders, I ask them:

> *If mice are so different from us that they cannot suffer from mental disorders, then what is the point of developing drugs in mice to cure mental disorders of humans?*

For people who are using these terms, "lower" simply means "less like humans," and "higher" means "more like humans." This terminology is tied in with the incorrect view of evolution as a ladder of progress toward especially evolved beings, such as humans. How would animals, used by humans for biomedical research, classify the human species? Of a high order? Crown of creation? Very unlikely!

An animal species cannot be considered of a relatively "higher" or "lower" order on any scientific ground, because the idea of "lower" and "higher" is just a concept that does not reflect reality. We classify different animal species into a higher or lower order, depending on our personal, hence subjective relationship with these species. This view puts all animals commonly regarded as vermin or pests into the lowest order—e.g., mice and rats—and those animals who have a charismatic appeal, because we know them as companion animals—e.g., dogs, cats, rabbits, hamsters and guinea pigs—into a higher order. Finally, we put animals who look and behave in ways that are similar to humans—e.g., monkeys and apes—into the highest order. The fallacy in this categorization is that it does not help us determine whether one species suffers more during a certain experimental procedure (and hence deserves more of our concern) than another species. Unfortunately, even professional animal care guides use these unscientific terms of "lower" versus "higher" order animal species. I did a "Google search" on the exact wording "higher species," and my first hit was the Canadian Council of Animal Care (1997), one of the most renowned resources on laboratory animal science. Here is the statement:

> *The creation of transgenic animals is resulting in a shift from the use of higher order species to lower order species, and is also affecting the numbers of animals used….An example of the replacement of higher species by lower species is the possibility to develop disease models in mice rather than using dogs or non-human primates.*

This document does not elaborate on what scientific ground mice are categorized as a "lower" species that implicitly deserves less animal welfare concern than the "higher" species of dogs or non-human primates. The fact that rats and mice

are commonly considered of lowest order has probably allowed US legislators to explicitly exclude rats and mice in the legal definition of the term "animal," thereby negating the two most commonly used research animals' legal protection of their basic welfare requirements (United States Department of Agriculture, 2002). This begs the question:

> *What is the point of having animal welfare legislation if it does not protect the great majority (>90 percent) of research animals?*

I think *all* animals deserve the *same* consideration, whether they are a rat or mouse—of presumed low order—or a dog or monkey—of presumed higher order. It seems strange to me to categorize animals into different orders and then treat them accordingly.

Do those of you who work on a daily basis with different species in the research lab, feel that the degree of discomfort and distress experienced in the artificial living quarters and during standard procedures differ significantly between species of alleged higher versus lower order?

In my daily work with rabbits, rats, mice, hamsters and guinea pigs, I do not see species differences in the animals' reaction to discomfort and distress. When you ask if it is less distressing for a mouse than for a dog or for a monkey to be killed, I think there is no difference. If there is a difference, it is probably due to the person who does the killing.

Working with quite a number of different species, I have found that the prey species—such as rodents and rabbits—tend to be more distressed during enforced handling and restraint than predator species—such as dogs and cats. All rodents are distressed when they are kept alone, perhaps not to the same degree as dogs or monkeys, but they *are* distressed nonetheless. To this very day, I feel for every rat, mouse and guinea pig who had to live in our facility without contact with another companion. Frogs do not give the impression of being distressed in their living quarters, but they seem to be just as distressed as warm-blooded animals are when they are handled by people.

Many years ago, I worked with macaques and rats who were kept alone in barren cages. Both the single-caged rat and the single-caged monkey, were miserable—depressed and bored—and I must admit, I could not tell a difference in the degree of distress that they experienced. I have the feeling that even though we may categorize them as animals of "lower order" versus "higher order," rats and monkeys do not differ in their observable distress response to being permanently housed alone in boring living quarters. These animals were often restrained by humans for procedures. While the monkeys always resisted and gave the impression of being scared whenever they were restrained, the rats seemed to tolerate the procedure. The observer got the impression that being restrained was a much more distressing experience for monkeys than for rats. However, there is no reason to believe that this particular difference is somehow related to monkeys being more evolved than rats. The fact that rats do

not show their distress during restraint is probably a biological trick that increases their chances of not being killed by a predator who has caught them. Being forcibly restrained is probably equally distressing for *all* animal species, but some show it while others don't—for biologically sound reasons.

How useful is the concept of genetic relatedness in terms of animal care and welfare? Does the genetic relatedness of animals with us, the human species, affect our concern for their well-being and our willingness to care for their welfare while they are used for research, and when they are no longer used for research?

It can be a little dangerous to suggest that a particular species deserves better care than another—for whatever conceptual reasons—because it implies that this species (for example, chimpanzees) is more capable of suffering than another species (for example, rats). This belief reinforces the misconceptions of those who might wish to protect nonhuman primates, cats and dogs, but *not* mice and rats. Genetic relatedness should have nothing to do with our welfare concerns for animals. Suffering is a subjective experience, and it is therefore impossible for us to know how another organism is suffering. It might be easier for us to appreciate that an animal is suffering in more genetically related species—e.g., monkeys—because they behave similarly to us, but it does not necessarily mean that a genetically less related species, such as rats, cannot suffer similarly as we do, or as monkeys do. We just don't know, and as long as this is the case, we must assume that suffering is a universal phenomenon that may vary from species to species and between individuals of the same species, but which is experienced as unpleasant by all animals—including humans—independent of their genetic relatedness.

I believe that humans, other mammals and all vertebrates are capable of suffering, but what about invertebrates? Some are probably suffering, but I cannot imagine an amoeba does. So, where do we draw the line and stop worrying about suffering?

There *are* questions that are out of our reach, yet this does not imply that I disregard the fact that invertebrates are life forms and, when I observe them a little bit closer, I will quickly find out that all, including the amoeba, avoid "dangerous" situations, and that none of them wants to be killed. So, I try not to kill them consciously and without a "good" reason, e.g., ending the incurable suffering of an animal.

I do not believe we should be using something as vague as genetic similarity to determine how an animal should be cared for. I care for all animals with the same concern for their well-being. Whether they are rats or primates, they all deserve optimal care. Humans share about 40 percent of their genome with bananas, and 85 percent with mice. If this is the case, do we give 98 percent of our welfare concerns to chimps, with whom we share 98 percent of our genome, 85 percent of our welfare concerns to mice and 40 percent to bananas? Are we twice as worried about the welfare of mice as we are about bananas?

It seems absurd to use a human mind-created concept—such as genetic relatedness—as a guide for one's degree of compassion for an animal of another species, yet it seems that we tend to be more casual, focusing more on human concerns than the concern of the animal subject when we design living quarters and develop handling techniques for mice versus monkeys. Why?

I have the uneasy feeling that genetic relatedness with the human species is just a pretext, while money is the actual cause for our relatively discriminating treatment of mice. After all, it is much more expensive to care for one monkey than for 100 mice, and it is much more expensive to replace one monkey than 100 mice. Perhaps this is the main reason why we tend to be more responsible when doing research with monkeys versus mice, i.e., animals who are genetically related to us, versus animals who are less related to us.

To classify animals into those of higher versus lower order, or to classify animals according to their genetic relatedness to the human species may have theoretical value, but it would be unscientific to use these concepts to determine the relative importance of the respective animals' welfare needs.

2.3. Human-Animal Relationship

2.3.1. Affection for Animals

Should animal care personnel be encouraged to establish and foster affectionate rather than neutral relationships with the animals in their charge?

Animal care personnel and researchers should be encouraged to develop affectionate relationships with their animals. Having such a relationship assures that you regard the animals as living beings, rather than biological test tubes. As such, you will be more careful and more patient. You will think more about what the experimental procedure implies to the animals. You will get more creative in refining procedures that are normally stressful or distressing to the animals. You will thus enhance their well-being and, by doing so, you will increase the scientific validity of the research results.

I became a vet tech because of my love for animals. I chose this job because the animals here are in need of someone who cares about them, and not so much because of the research data they provide. If I can make the life of just one of the animals under my care more comfortable and possibly more enjoyable, it is worth all the effort to me. We all grapple with this same issue:

> *We love animals, yet we work in an environment where animals are often subjected to quite terrible situations. But because we love the animals, we are a guarantee for them that they will receive from us the best care possible.*

Many of us will rejoice when animals are no longer required for research purposes and will gladly seek another profession at that time. Until then, the animals need us!

When asked how she deals with attachment to animals in her care, a veterinary technician gave the following answer for the journal *Lab Animal* (Anonymous, 2006):

> *It's hard because I am passionate about what I do and because our animals are long-term. It is important to be attached and there are certainly days when I am in tears, but I think if I ever felt unaffected by euthanizing our animals, it would be time for me to leave. As hard as it is to be passionate about what I do, I think it is a serious job requirement.*

Even the American Association for Laboratory Animal Science (2001) concedes that:

> *The bond between people and animals in the laboratory, if understood and used consistently, can minimize certain variables related to stress in the animals.*

And Herzog (2002) elaborates that:

> *There is every reason to believe that individuals who care about their wards on a personal level actually treat the animals better. Inevitably, individuals who work with animals in the context of biomedical and behavioral research will sometimes form bonds with the animals with whom they interact. Although human-research animal relationships may enhance the well-being of laboratory animals, they involve a moral cost to the human caretakers. Institutions should acknowledge the existence of these bonds and provide support mechanisms to help laboratory personnel deal with the moral challenges of their profession.*

I agree wholeheartedly that developing a close bond with research animals can only be a good thing. It seems to me that we can easily get hung up on trying to divorce our emotions from objectivity. I don't think that any normally functioning human being in the world does anything for any reason other than emotional. Is it not the premise of all biomedical and ethological research to make human and animal lives better? If you want to make lives better, it's because of emotion, not because you are logically attached to life. I feel empathy for my animals, and I am genuinely concerned about their well-being, otherwise I would probably not notice when an animal is not behaving and responding normally because of a developing health problem.

For some people, it may be defense mechanism not to get too attached to animals who are intended to be killed within a short time. Wouldn't it be unbearable for technicians to euthanize hundreds or thousands of mice—sometimes after having had to make these animals ill and suffer—during a work year, if they were emotionally attached to each and everyone of these mice? As a researcher, I do take the animals' welfare very seriously and get terribly upset if they suffer, even though I don't have an affectionate relation with them. It's perhaps not necessary to develop affectionate

Robert Dodsworth

Robert Dodsworth

Figure 1a,b

If you are on good terms with the animals in your charge—here a rhesus macaque—they will show their trust by engaging in affectionate social interactions with you, such as grooming (a) and allowing you to groom them (b).

relationships, but kindness toward animals should be a professional prerequisite for any person who is hired to care for animals in research labs. We owe this to the animals!

We also owe this to ourselves, because when we are not kind to animals, we are also not kind to ourselves and to other people. How can we ever expect to find happiness when we are not kind?

If you are not kind to your animals, make no attempt to enrich their boring, often depressing living quarters by addressing species-typical behavioral and social needs, and never show any kind of affection toward them (for example, by offering them food treats from time to time), then I really don't think that you should work in an animal research laboratory. Unfortunately, I did and still do find such

people in animal quarters, so if anyone is offended, well, you just might be guilty! Being indifferent, inconsiderate and rude has no place in a lab, zoo, or anywhere, for that matter.

For me, developing affectionate relationships with the monkeys in my charge (Figure 1a,b) is always a spontaneous process. I know that I could develop this sort of rapport with other species, but based on my experience with mice, I do wonder whether there is a size limit.

The way we handle mice is not very attractive! It would never cross my mind to lift a larger animal by the tail or scruff, essentially ignoring whether or not he or she is cooperative. I suppose we handle mice the way we do simply because they are so small. I am really wondering as I look at a picture showing a huge human hand grabbing a tiny mouse baby by the scruff! For an animal that small and vulnerable, the evolutionary programming might very well be "Live as if there was always somebody wanting to eat you." Is it still possible to establish a relationship of trust with mice, in which they will come to you and enjoy being with you, and in which you can exchange signs of affection?

Yes, it is entirely possible to establish a close relationship with mice, involving trust, petting, and lots of physical contact. This is done with rats all the time, and the two species are not that different. The problem with mice is that most of the ones we're likely to come into contact with are wild. I have live-trapped hundreds of deer mice in my house and have never been able to turn them or their offspring into pets. You just have too many generations of skittishness bred into them. On the other hand, one of the best pets I ever had was a store-bought mouse. He was pure black and his name was *Juarez*. He lived in a small cage with a wheel, and he loved to come out every evening for some cuddle time and hand feeding. He was as tame as a dog—very responsive in every way.

We had a wild mouse spending two winters in our home. She would appear in the fall and make her way out again in spring. Each evening, we could watch her from our pillows as she explored the desk in which she had also built her nest. There was no way for us *not* to get to know this critter very well, and the naming happened automatically. So for us, this tiny little mouse was not just a mouse, but *Minette*. I am telling this story to make the point that the development of an affectionate relationship with animals does not necessarily depend on their evolutionary relatedness with our own species or on their size, instead, it may well be a function of the amount of time we spend observing individual subjects, and by doing so, discover their uniqueness. Rats and mice *are* very charismatic when you deal directly with them, rather than with the "idea" of them (Figure 2). My students often say things like, "Oh, they're actually rather cute" when finally coming face to face with these animals.

Larissa Lone

Figure 2

You just have to watch them closely to realize that "even mice" are charismatic animals, not just disposable test objects.

2.3.2. Giving Animals Names

Do you give names to the animals in your charge?

Naming the animals helps me realize that I am working with sentient beings who deserve my consideration of their well-being. It is probably more difficult to be callous toward a monkey who is called *John* than to a monkey referred to as ID #79045. As a clinical veterinarian, I observed that nonhuman primate-caregivers became markedly more concerned for and interested in the animals in their charge when the ID number tags on the cages were replaced with name tags. I guess we can all relate much better to names than to numbers, and we tend to treat named versus numbered animals accordingly. The naming of animals in research labs could serve as a safeguard for optimal animal care.

I was encouraged not to assign names to the many rhesus monkeys in my charge. I was admonished that the animals are research subjects, not pets. The concern was that having names for the animals might blur this distinction between a research subject and a pet. It did not seem possible to remain distant—emotionally isolated—from the animals. In fact, the inevitable closeness that resulted from those intimate interactions was precisely what made us capable of doing what we were asked to do. Eventually, we all came to know that F49 was Sam, A12 was Rosie, and Z13 was Curious. Such attachments are the results of compassionate people doing their job right (Wolfle, 2002).

We have an investigator who is against the naming of rabbits assigned to her research protocol. The PI (principal investigator) is afraid that, when bonding with her research subjects, we add a variable that is detrimental to performing research. Our staff feels that this is an antiquated mentality and we are standing strong in our position of naming all animals in our charge!

I have run into that same mentality here, but ended up naming the animals anyway, using their ID numbers only for the records. I name our animals primarily because we have so many of them, and it helps our care staff and me keep better track of who is who. We have monkeys, cats, rats, rabbits and mice. All of them, except the mice, have their names. We have a high turnover of mice, and this makes the name-giving a bit of a challenge, but we name the mice who stay around for a while.

Giving names can cause methodological problems under certain circumstances. I remember a large breeding group of rhesus macaques who was constantly tyrannized by the beta-female and her female ally. The beta-female was so vicious that I gave her the name *Devil*—her official ID was t-42. The situation became serious and I finally had to remove *Devil* and her buddy to restore the group's harmony. If I had assigned *Devil* to an ethological study and done the observations myself, my perception would have been pre-conditioned, probably not so much by the name *Devil* but by the experience I had with that particular animal. Knowing that *Devil* is vicious, I would presumably put my attention first on her before anybody else—for instance, if a group member screamed during a dispute. In this manner, *Devil* may end up being scored as the most aggressive animal of the group, which she actually was not, because I have unintentionally missed many overt aggressive acts from other animals.

I think that it is not really the name *Devil* that would have influenced your attention but the actual experience you had with this animal. You cannot avoid such experiences, so your focus of perception is bound to be pre-determined by memory. This is unavoidable regardless of whether we give the animal names or go by their IDs.

2.3.3. Touching Animals

When you work with animals on a regular basis, you may develop an attachment to certain individuals and then want to touch, stroke or groom them. This is a very nice experience, but it can be dangerous if you misunderstand the subject's feelings and motivations. How do you know for sure—and you must be sure for your own safety!—that an animal *wants* to be touched, stroked or groomed by you?

2.3.3.1. Rodents

If a **rat** enjoys being groomed by me, she will respond with a relaxed stance and closed eyes, and then she will also start grooming my hand.

A few years ago, we had a small litter of **mice** who lost their mother when they were only 12 to 15 days old. They were without a mother for almost two days. I was successful in caring for three of them to the point that they thrived. Because

of the stressful event of losing their mother before weaning, they were not suitable for research purposes. They became my "sentinels," really my pets kept at work! I would handle them a little more during cage changing than the other mice, but not usually between cage changing. Over time, the male mouse came to accept my petting. He no longer moved away but seemed to be completely at ease with the situation. His two sisters were different. They did not like the gentle head rubs and always tried to get away the moment I touched them.

I often had the chance to hold **guinea pigs** in my hands but never got the feedback from the animals that being gently stroked was appreciated. The animals would remain still and would never contact-vocalize in the typical guinea pig fashion; they showed no reaction to being petted. Adult guinea pigs never groom each other, so it is probably not such a great experience for them to be petted by a human.

Hamsters and **rabbits** demonstrate very clearly that they do not enjoy being touched: they try to get away from my hand.

2.3.3.2. Monkeys

In our **aotus monkey** colony, we have a few animals who will back up to the front of their cages to get a good back-scratch. If you stand in front of their cages, stick your fingers up and do the scratching motion, they will back up, and you can see on their faces that they enjoy it when you groom them. When they have had enough, they just leave.

When a **rhesus monkey** approaches me and does a rump or chest present, I can tell the animal wants attention. Typically, a monkey will press his or her body up against the cage, allowing me to gently tug at the fur from the outside of the cage, as if I were grooming. I am sure the animals enjoy this as much as I do.

I have one girl, *Meera*, who loves to have her bum rubbed and her face groomed. She actually asks for it by presenting herself. This is a very clear signal that the animal is not afraid of you and wants you to come closer and, as in this case, start a grooming session. We had another monk [monkey], *Kiwi*, who absolutely loved human contact. She would pretend to be asleep after a procedure, so that I would hold her longer before placing her in the recovery cage. I used to watch her squint her eyes slightly open to see what was going on, only to quickly close them if someone was looking at her!

The key signal that tells me that an animal likes to be touched is when she or he "presents," i.e., entices me to do so. A chair-restrained rhesus monkey, for example, will twist her body in an attempt to present her rear, thereby letting me know that she wants to be groomed. Under such a condition, the monkey will show no fear or aggressive-defense reactions, but rather be relaxed and calm.

I know quite a number of rhesus macaques who will present their chests, only to get very mad when you touch them. Maybe presentation is not always a reliable sign that an animal wants to be scratched?

What you describe is a quite common scene in primate labs. I believe that, what the animals are doing is nothing less than teasing us. These guys are bored in their cages—who wouldn't be?!—and they are looking for some action. They present their chests or rear ends to the naïve caretaker or visitor, knowing beforehand what the reaction and the outcome of this little game will be. You have hardly touched them, and they will turn around, bang against the cage wall like a devil and/or threat-yawning like a lion. You may be shocked and react accordingly, and that's what they are after: your reaction to their display. Your reaction will reinforce the teasing. Once you no longer participate in this game, you will no longer be invited to groom, but you can pass those animals without being harassed.

A human-animal relationship that involves contact is very rewarding for both human and animal, and it helps to instill and foster trust. As such, developing such a relationship with macaques seems worthwhile as long as it is done carefully. It is usually fairly evident which animals are soliciting grooming simply to tease you and which ones really want to be groomed.

2.3.3.3. Cats

Cats are a bit tricky when it comes to trust. They can easily give you the wrong impression of enjoying being touched. Their time span for direct continuous social contact is usually very short when compared, for example, with dogs and monkeys. When you have reached this time limit, you may be in for a hiss or even a scratch. These critters can switch from "I am in bliss while you groom me!" to "Let me alone!" in a blink of an eye. I have had encounters with cats during which they allowed me to touch them, and then all at once, without any warning, turned around and gave me a swat. In some cases, the animal will solicit to be touched again right after swatting.

This exact situation happened to me just yesterday. I was at a friend's house and her cat entered he room. The cat knows me well and jumped straight on to my lap. He settled down, began purring and kneading, and seemed very comfortable with my stroking him. After a couple of minutes, my attention was distracted and I looked away, and at that very moment my hand was suddenly attacked, quite viciously! I think cats might be a special case, because they are generally solitary but live in groups when there is plenty of food available. Perhaps they have not lived socially long enough to have evolved a gesture to say "Thanks for the strokes, but I've had enough," other than by hissing.

2.3.3.4. Farm Animals

One of the bull **calves** in my charge looks forward to a daily "sponge bath." When I approach his stall, he gets up, sticks his head out and watches me until I come over. He


Viktor Reinhardt

Figure 3

Most social animals like to be touched, provided they trust you and can initiate and terminate the interaction at their own wills.

constantly rubs on me while I am wiping him down with a damp cloth. I have no doubt that he likes it very much. If you gangbust calves and go to them for petting, they are usually fearful and combative, but if you give them the space to make their own decision when to approach you, then you can scratch them. They seem to truly enjoy this and often end up being quite affectionate (Figure 3).

I have worked many years with **pigs** and can affirm that they do enjoy human touch very much, but it must be *their* idea, and they will of course let you know when it should end, usually by moving away or vocalizing if they feel trapped. I purchased a toilet brush for scratching the pigs in my charge. Most of them cannot resist, once they realize what it is for and how good it feels. They seem to like being scratched just about anywhere. When I need to obtain a rectal temperature, I scratch them around their tails. They like this and stand still, allowing me to get their temperature without any ado.

2.3.3.5. Cold-Blooded Animals

When we had tree **frogs** in our home, I would often gently "tickle" one of those little guys under the chin. The frog would be transfixed—as they often seem to be—but I couldn't figure out if the animal was blissed- or stressed-out.

My daughter has a leopard **gecko** who is very responsive to human interaction, and it is obvious that he prefers her to anyone else. If I am holding him and she

comes near, he literally jumps to her. He tracks her voice and likes to crawl up on her shoulder. As for touching, he will tolerate his chin being rubbed, but other than that he doesn't like to be "petted."

I have had a female green **iguana** as a pet for seven years. It is my impression that she loves it when I rub her neck or gently scratch her back; she closes her eyes and leans into my hand, almost like a dog.

I had an Oscar **fish** who *loved* to be rubbed on his stomach/ventral area. *Oscar* was trained to jump, roll over and move from point A to point B. He was used for training/teaching purposes only, but became very attached to certain caregivers, i.e., would only eat if fed by them and would even allow them to rub his stomach. He was great with the students because he opened their eyes to the idea that fish have more cognitive abilities than most people give them credit for. I don't know if *Oscar* was an anomaly, but I am willing to bet he wasn't.

When I was a boy, I used to touch trout in our river. I would reach under one of those typical overhanging rocks and very carefully find my way to a trout. Gently stroking her belly with my fingers would inevitably make her stay still.

2.3.4. Alleviating Fear of Humans

Does gentle, regular interaction with humans help animal subjects overcome their fear of humans when they are handled during experimental procedures?

I firmly believe that regular interaction helps animals overcome their fear of humans and procedures. Several years ago, I worked on studies with **rhesus**, in which we were told that the animals would become very ill and require great care from all the techs in order to keep them comfortable. Because we were doing terminal studies, we generally received "recycled" animals. Many of them were quite afraid of humans when they arrived in our lab, but we were instructed to spend time with them so that they would become used to our presence and develop a bit of trust in all these hairless apes. We would sit by their cages, give them treats, and try to desensitize them to human contact. The time we spent proved to be very beneficial when we would have to care for these animals later during the actual study. I will admit that we didn't have a 100 percent success rate, but quite a number of animals would would seek our attention and affection after a while. I vividly remember an adult male cyno who would raise his arms up, much like a small child, to be lifted from his cage to the examination table for treatment. Following treatment he would cry if placed into his cage immediately, because he wanted to spend a little more time outside, being held or groomed by one of the techs.

I have fostered a relationship with some of the rhesus macaques in my charge, strong enough for reciprocal grooming (Figure 1a,b). Animals with whom you have this kind of affiliation are more apt to cooperate under routine husbandry circumstances such as catching, weighing and TB testing. Having such a close bond with one of our

Viktor Reinhardt

Figure 4
Animals who are visited regularly by research personnel gradually lose their fear of people.

rhesus girls helped me tremendously when it came to "doing business" with her. *Kia* was a very friendly monkey and liked pretty much everyone. Because I worked with her every day and was the one feeding and playing with her, I like to think that she had a particularly strong bond with me. On a few occasions, she escaped, and I was able to walk into the room, scoop her up and place her back in her cage. During chair training, she would snuggle into me—like a child hugging her mother—and we would sit in front of the chair. I would put treats all over the chair and she would retrieve them without leaving my side. She was a doll when I had to give her injections. There was no need to squeeze her, I only had to show her the syringe and she would back up close to the front of the cage and allow me to proceed with the injection. I had not trained her to cooperate: she just did it spontaneously. I visited *Kia* several times a day and we would often groom each other.

Some of the **marmosets** I deal with will groom the back of my hand if I "present" it to them, or they will jump onto my shoulder and groom my hair or neck. One particular marmoset even tries to pry my lips open to "groom" my teeth—but, yes, there are limits! The marmosets with whom I have a grooming relationship don't have to be physically restrained during common procedures, probably because they don't see my hand moving in their direction as a "threat."

Caged macaques often freak out when a person dressed in professional protection garb is entering their room. When such a person wears heavy leather gloves, things get really wild! What can we do to help the animals deal with their negative experience-conditioned fear of people?

Several things seem to help our monks:

1) The animal care staff and the researchers are in and out of the rooms

frequently, at least once every two hours. Our researchers are very good about just visiting their monkeys (Figure 4).

2) We try to make each visit not a frightening experience for the animals. We remain quiet and avoid sudden, jerky movements that could alarm the monkeys.
3) Low-level background music is played all day long in the monkey rooms.
4) If one monkey is being sedated via intramuscular injection and taken out of the room, we give a food treat to *each* of the other animals of this room.

Our monks are pretty good about not freaking out when someone enters the room.

It is great that your researchers are taking the time to visit the monkeys. If more investigators would be inspired to do this, less negative conditioning would probably occur, because the animals would learn through experience that the researcher is usually harmless, not a life-threatening enemy.

2.3.5. Summary and Conclusions

Relationships that develop between facility personnel and laboratory animals may result in an overall reduction in stress for the animals, and they may serve to buffer the potential stress of certain experimental situations. Administrators of animal research, testing, and teaching programs should look for opportunities to encourage the development and maintenance of bonds between personnel and laboratory animals, beginning with the initial employee interview (Bayne, 2002). Researchers must continue to question the barriers that have traditionally been erected against forming human-animal bonds in the name of objectivity and to investigate seriously the ways in which fostering the formation of such close relationships can promote animal welfare without compromising the scientific respectability of research (Russow, 2002). Naming animals helps to correctly and quickly recognize individuals. An affectionate relationship based on mutual trust often makes it possible to touch or groom an animal. Certain postures and gestures indicate whether an animal likes to be touched. The fear of humans can be alleviated by visiting animals with good intentions on a regular basis.

2.4. Emotionality—Is it Unprofessional to Cry?

Working with animals for researchers can sometimes be very stressful, hectic and frustrating. Is it justified to cry at work once in a while?

If I cry due to work-related issues, I just remove myself from everyone. If anyone notices my emotions, it is labeled as unprofessional.

Are you joking? I cry at work all the time! More seriously, crying at work for the

animals can mean that your empathic feelings are alert, rather than put to sleep by the routine of the lab work. So, to me, it is a healthy response to an emotionally upsetting situation. I would ask those who are uncomfortable with those of us who cry to just let us be, especially if it is not interfering with our work. Crying is an important safety valve that some of us need. I do the termination of my macaques, because I want them to have the feeling that this day is not different from those when they are normally anesthetized. Some of these animals I have worked with weekly, if not daily, for up to five years. That loss deserves some tears! I believe in the research that is done with the animals, but this does not hinder me from offering them the best possible care, and I will cry when they are gone.

I have also cried at work on occasions when an animal suffered unnecessarily, or when I was involved in putting down an animal I had worked with for a long time. Expressing one's sadness is only unprofessional if it prevents me from doing a job in a way that is best for the animal. Otherwise it is simply an indication that I have compassion. One of the most horrible times I ever had at work was when we traded out one dog for another, who would be used for a terminal study. We had received a group of dogs from a class B dealer, and a huge beautiful golden retriever pup who we named *Anton* was one of them. It was decided that if we could find a trade with the dogs we already had, we would save *Anton*. When my supervisor brought the little female terrier mix over for the trade, we just broke down. We used her because she would never be able to be adopted out due to the tick-borne disease research she had been used for. She was so sweet! I sat there for a good 30 minutes crying and talking to her and hoping someday she would forgive us. On the positive side, Anton is running on the prairie with a loving family!

Whenever an animal had to be put down, be it mouse or dog, my supervisor was very strict about respecting the animals' dignity. If *anyone* joked or kidded about it, she was like a cobra to correct them as to why it's no laughing matter. She is still my dear friend! We do such a tough job, especially, since I think all of us are animal lovers. If we didn't cry, we would probably also not care, and wouldn't feel bad about what we are doing. If I didn't cry in the face of the animals' suffering, I wouldn't be in this profession. I, too, have hid in order to be alone, because crying is looked down upon here. I do support the research, but I am also sad that there isn't yet another way besides using animals to accomplish the goals of this research.

I am glad—as hard as it is on a daily basis—to work as an animal technician, because I feel that I can offer the animals a special gift. Every day, I do whatever I can to foster their well-being and make sure that while they are here, they are getting the best care possible—and the best toys, of course! After working with a group of beagles for several weeks, I was asked to assist in the euthanasia of my favorite one, whom we had nicknamed *Cico*; he was a porker but so cute! I did assist, but I cried like a baby. My co-workers' solution was that I should not be around for future euthanasia. But this was not the point. Even if I wasn't there for the euthanasia, I still would have been upset; it's hard not to be. I did and can do the euthanasia; that's part of my job. I have

not had to say goodbye to any of our monkeys yet, and I honestly don't know how I'll deal with this situation.

Yes, I think it's absolutely normal to be sad and cry sometimes with our line of work. To bottle up feelings of sadness, frustration or anger doesn't change the unacceptable situation but drains your energy and enthusiasm and makes you bitter. Walking through animals rooms, with row after row of cages from which lonely monkeys were sadly looking at me, made me often cry because of my limited power to change the situation. It's hard to be exposed to these realities. Crying is certainly a more healthy response than angrily arguing with investigators or administrators who are responsible for the situation. The first response gives you some relief, the second makes you even more frustrated, tense and angry—because you are usually talking to deaf ears.

People often make the mistake of assuming that we must not be animal lovers, because we work in biomedical facilities. I think it's just the opposite. Most of the animal technicians and animal caregivers I know truly do love animals, and I think this is the main reason why we chose to work for the animals in research labs: we can make life easier for the animals in our charge. Yes, there are some days that are almost unbearable, but I know that I do make a difference for the animals, and this is what keeps me from running away. The animals need me!

I work with guinea pigs, and we euthanize quite often in order to collect tissues. I haven't cried yet, maybe because I don't spend much time with individual animals. Although I don't cry, each euthanasia hurts! I'm not a crier normally, but I do go through times of depression. I can't tell you about my experiences of loss and sorrow both for personal and professional reasons, but I can say that the feelings we have for the animals are an important part of what makes us the best candidates for our field. Imagine if we had no feelings for the animals we work with. Things would be horribly different. Try to keep in mind what you give them and why you are important to them. It is the nature of this field that makes it difficult, but through love and commitment, we are able to lessen the burden on the animals.

We too had to let some of our guys [macaques] go off to a better place. Although no one would express any tears, there would definitely be a different feel in the air. Some became quiet, others a little snappy, and others would choose to just not be around for the terminal procedure. As for myself, the day before, I would sit with "my young man" or "little girl" and talk to them. I'd let them know that I was happy for them to move on, and thank them and apologize for the sacrifice they have done for us. It is embarrassing to cry at work. Therefore I don't, but I've come close! I will always think of the monkeys I have had the privilege to work with, and I will always talk about them and tell stories about them for a long time.

A month ago, we had a young monkey experience a seizure after she was used for an experiment. After the seizure had stopped, she was paralyzed completely on her left side. She was awake, alert and hungry. Every time I tried to give her some food, she made an earnest attempt to sit up, but invariably would flop all over the place. It

was heartbreaking to witness this, and I couldn't help but cry. Here was this perfectly healthy animal, and we did this to her! Because I work with the girls on a daily basis, I can't help but become attached to them. I can't work any other way. I know what I'm in for, what they're here for and what will eventually happen to them. I'm fortunate to have an understanding boss. When the time comes to sacrifice an animal, I will inject the anesthetic and that's the last I see of the animal: a sleeping peaceful monkey. It is comforting to know that I am not the only one who gets upset with much of what we do and that there are other people with whom I can share my feelings.

In conclusion, it is not unprofessional to cry when you face situations in which animals have to be killed or endure unnecessary discomfort, pain or distress while you are helpless to interfere on their behalf. The expression of sadness in such situations is a reflection of your sincere concern for the well-being of animals.

2.5. Humane—What Does this Term Mean?

How would you define the word "humane" in the context of animals in research institutions?

I think the term "humane" should be defined as "to treat animals the same way you would treat a human being," that is to say, with respect and concern for their well-being.

If only you were right! Humans very often treat humans even worse than they treat animals. Those who have obtained a certain power position—be it social, economical, political or sheer muscle strength—often misuse their power and treat other humans in ways they would not like to be treated themselves. It is a very sad reality. I think the term "humane" implies an idealistic vision, yet we do need a working definition because it is used in legal animal welfare texts such as the US Department of Agriculture's Specifications for the "Humane" Handling, Care, Treatment and Transportation of selected animal species (United States Department of Agriculture, 2002). The term "humane" is not defined in these regulations, leaving it up to the research industry to interpret it as deemed practicable. The National Research Council claims that the goal of its Guide for the Care and Use of Laboratory Animals is to promote the "humane" care of animals used in research, but fails to explain what this quasi-noble term actually means (National Research Council, 1996). Not being defined, the term "humane" has no value and lends itself to subtle and gross misinterpretation when used in animal welfare legislation and guidelines.

I am not sure I can tell you what the "humane" treatment of research animals means to me. Our chosen field of practice tends to skew our view of the plight of research animals. We amputate their toes for identification, we cut their tails for genetic analysis, we burn them to study healing, and we subject them to chemicals to see how these harm them. We have many, many ways of causing them harm to study abnormal

occurrences in humans, and then we "sacrifice" them at the end of a study. This does not sound very "humane" to me, but who am I to judge.

If we do all these "inhumane" things to animals, don't we have to "judge," i.e., make an ethical assessment so that we can live at ease with ourselves? If this ethical assessment puts us at dis-ease with ourselves, we will do something—for example alleviate or avoid the pain or distress of other creatures—to come back to a state of mental and emotional ease.

2.6. Euphemism—Do We Really "Sacrifice" Animals?

How appropriate is it not to use the verb "killing" when we euthanize an animal at the termination of a study or because the animal is no longer of use for biomedical research?

Many times, I got in trouble for writing in a protocol or report that the animals will be "killed" at the end of the study. In the interest of clarity and honesty, I always put "killed" in the first draft, but it is inevitably changed by someone higher up the chain to "euthanized" or "culled." It seems to me that it would be more honest to stick with the facts. When it comes to terminating an animal's life, euphemism is a cheap way of beating around the bush. The word "sacrifice" implies that the act of killing is "sacred" [justified] and performed by a "priest" [the scientist], and that the subject is "offered to a deity" [science]. This euphemism is a gross distortion of reality. Things are much more "down to earth" than this: we "kill" the animals!

The principal investigator who kills animals—or has others do the killing on his or her behalf—to achieve the goal of his or her scientific endeavor probably feels more at ease when he or she can hide behind the elegant phrase: "I have sacrificed animals for an important scientific project." This kind of wording is not "scientific," because it has nothing to do with the reality as experienced by the animal who, de facto, is killed.

To me, the word "killing" paints a picture of violence, so I prefer to use the verb "euthanizing," because it makes me feel somehow better about the death and loss that I face daily. It can at times be overwhelming! "Terminating" is also accurate but carries the same coldness as "killing." When I have to euthanize an animal, I am not callous, but do it in the most humane way possible.

I agree, "to euthanize" seems to be the most appropriate verb, however, it may be misleading in regards to animals who are "killed" because they are surplus. When watching hundreds of "surplus rats" being killed or gassed in big tanks, because they are "spent," have the "wrong sex," have the "wrong phenotype," or have reached the "end of research," I don't feel the word "euthanasia" is appropriate. I am not so certain that rodents killed with gas as a way of "inducing death without pain" do not experience distress—either in theory or in practice. I believe the majority of them are

in panic and feel considerable pain before they get unconscious, particularly in "bulk killing" settings.

If we use the terms "sacrificing" or "euthanizing" with full awareness of what they actually imply to animals in research, we are honest to ourselves and we will do our very best to minimize the discomfort, pain and distress that the animals may experience during this life-terminating procedure. If, however, we use these polite terms to give the impression that what we are doing is justified and humane, we are dishonest to ourselves and to the lay public.

2.7. Self-Awareness—Do Animals Recognize Themselves?

Some species we are working with respond to the reflection they perceive in a mirror. They may use a mirror to see objects that they could otherwise not see, and they may respond to the reflection of other conspecifics and of themselves differently. Do they recognize their own reflection as themselves?

It's funny that you have brought this up, because just the other day I was telling the story of such a case. In my first job, we had a cyno (long-tailed macaque, *Macaca fascicularis*) who, we firmly believed, was conscious of himself. He would use his small cage mirror to check areas of his face for grooming and to send facial expressions across the room at other animals. Our attending vet found this so delightful that he bought a large wall mirror and hung it across from the monkey's cage. We then all got a kick out of this animal using the mirror to examine and groom the fur of his back and to check his teeth very thoroughly. He must have recognized the reflection in the mirror as himself. If he had thought the reflection was another monkey, wouldn't he try to groom the mirror monkey rather than himself? He was a real character!

I worked with two rhesus females who would very attentively look into mirrors while grooming their *own* faces, especially around the eyes. To me it seems logical to conclude that these two monkeys made the connection between the reflection in the mirror and the sensation that went along with seeing their own faces being groomed: they saw themselves being groomed.

One of my cynos seems to recognize herself in the mirror. A few months ago, I first noticed *Annie* looking into the mirror and examining her own teeth. She used her fingers to pull her lip down to get a better look, with her face close to the mirror. She noticed a small raisin stuck to a tooth, used the mirror to direct her fingers to the raisin, picked at the raisin and finally removed it (Schultz, 2006). Recently I put a red dot on *Annie*'s forehead while she was anesthetized for a medical procedure. After she had recovered, I took her to the mirror. *Annie* put her face very close to the mirror and looked at the dot for some time. Then she reached up to the dot on her forehead—not in the mirror

image!—while looking into the mirror and tried to touch the dot on her forehead. On another occasion, I put a small piece of white sticky paper on top of *Annie*'s head. At the mirror once again, she noticed the white dot in her mirror image and removed it promptly. Later in the day, I saw *Annie* searching the top of her head with the help of the mirror. She appeared to be "checking herself out," looking for another dot!

I don't want to stretch this discussion too much beyond the mirror but would like to make this, perhaps provocative, statement that the members of any animal species that develops a social hierarchy, *must* be self-aware, otherwise no stable relationships, predictable for each group member, could ever evolve. Cattle, for example, establish dominance-subordinance relationships that are respected by the individual social partners for many years. I have no doubt that these animals have self-awareness, but this does not imply that individuals recognize themselves in a mirror as humans do. Different species have different perceptions, but they may nevertheless share the same mental faculty of self-awareness.

Would you include invertebrates such as bees and ants?

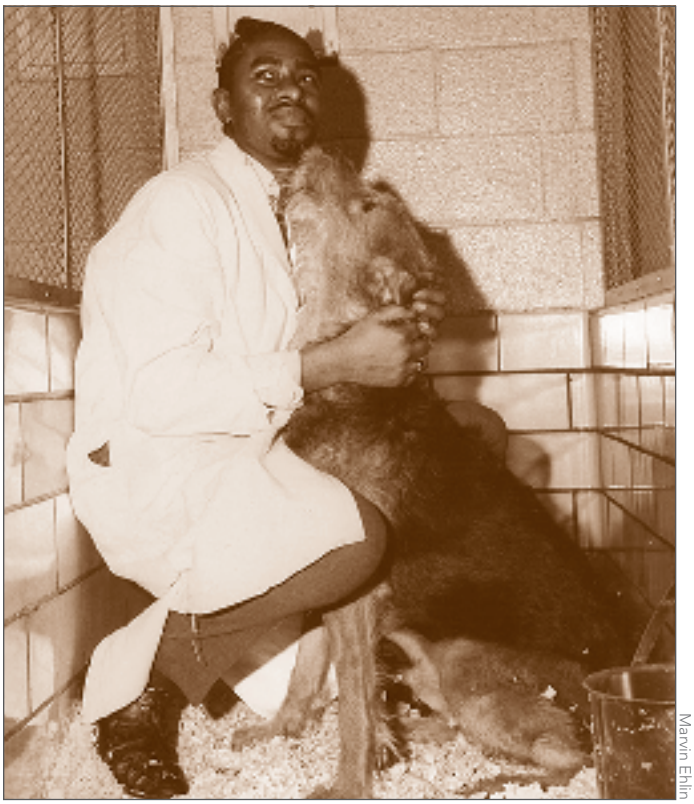
Yes, I would include bees and ants and any other creatures who do establish stable social one-on-one relationships. Just considering the highly sophisticated inter-individual relationships and communication skills of bees and ants, I have no doubts that individual members of such colonies do possess self-awareness, perhaps not the ego-dominated self-awareness of humans, but the pure self-awareness that is not linked to a memory-based personal story.

Empirical evidence and ethological considerations make it plausible that animals are capable of self-recognition.

2.8. Pound Dogs—How to Work with Them in the Research Laboratory

Is it emotionally more challenging to work with pound dogs than with dogs who have been bred specifically for biomedical research?

It is a lot more difficult for me to work with pound dogs, such as a golden retriever or a Labrador, than with the dogs who have been bred for research purposes. I know that the dogs from the pound were companion animals at some point. They exhibit many signs of a companion animal: knowing how to sit and give paw, wanting to play fetch with a toy, or just craving human attention (Figure 5). I can offer these pound animals, who have abruptly been turned into "research animals," some comfort by trying to recreate a home environment as much as I can while they are here. Because of my experience as a dog owner, it's easier for me to provide enrichment to ex-companion dogs than to purpose-bred dogs who are more aloof, although some do play.



Marvin Ehlin

Figure 5

Dogs from the pound often behave like companion animals and crave human affection. It can be an emotional challenge to work with them in a research lab setting.

I remember the time when we worked with dogs who were ex-pets. It was emotionally very disturbing, and all the techs and the majority of the researchers I worked with found this circumstance extremely difficult to tolerate, even though we knew that the owners had willingly sold their pets to our supplier. We were lucky in that the researchers used to turn a "blind" eye to our re-homing schemes and entered into the records that the animals for whom we found new homes had died from "natural" causes.

Since our facility has a fairly strong adoption program, I would rather that we use pound animals, as it gives these dogs a chance to be adopted into a good home. In addition, most pounds in the US hold animals for possible adoption only for about 5-7 days and then euthanize them. At my prior facility, we actually removed dogs from the pound's euthanasia area just prior to them being killed—literally minutes before. In the two years that I worked there, we were able to return four dogs to their owners. While that's not a big number, you have no idea how good it felt to bring these animals back to their original homes!

If research laboratories could purchase pound animals scheduled to be euthanized because no new home could be found for them in time, pounds would make enough

money to allow for a longer stay of ex-companion animals, thereby increasing their chances to finally get adopted. I would rather see dogs used for research purposes than killed in pounds. The hard part is convincing the "general public" that those animals to be used for research would be sold just prior to euthanasia and not at the whim of the people running the pound.

Animal lives could be saved if pound dogs who have not been adopted in time, were given to research labs rather than killed and another healthy purpose-bred dog used for research instead. Working with pound dogs can be very challenging because the animals often show typical pet-behaviors, thereby eliciting strong emotional attachment.

2.9. Adoption of Animals After Research Completion

2.9.1. Adoption by Private Homes

I was wondering if I could get some feedback regarding adoption of animals after research completion. We were able to get one dog adopted by an employee after the dog had chewed at an implanted probe. Our institution had to get lawyers involved and go through a bunch of red tape, but the dog is now finally "outside," living a normal life with a caring family. This ultimately positive experience made us ask ourselves, "Why can't we do this for more animals?" We currently have an investigator who would like to adopt one of his canine patients once the study is over. The dog could live a normal life. Can anybody share experiences on successful adoption programs?

In the Netherlands, there is an organization for re-homing animals that is also specialized in re-homing dogs and cats who have been research subjects. This organization has contact with biomedical institutions, and I believe uses standard ownership transfer contracts. The dog or cat who is no longer used for research is placed with a foster family for an observation period. If the animal readjusts to normal life, bonds with the new family and is healthy, he or she can be adopted permanently in the new home.

We have an adoption policy that was drafted with the advice of our lawyers. We primarily adopt out cats, but occasionally also rats, frogs and rabbits. We have adopters sign a release/waiver of liability before the animal goes out. All potential adopters are screened as carefully as possible. The cats and rabbits are spayed or neutered and deemed healthy by the veterinarian before they leave our facility. We haven't—knock on wood—had any major problems with these adoption procedures. I think there's a good publicity potential in running adoption programs: (a) The facility shows people that it is concerned about the animals, and (b) gives evidence that there is research that doesn't harm the animals but leaves them fit enough to carry on a normal life as pets outside the laboratory.

For more than a decade, the University of Florida College of Veterinary Medicine has allowed investigators to arrange for the adoption of animals who are no longer used in research. Di Gangi et al. (2006) surveyed 458 cats adopted over a period of six years and found that 91 percent of the animals were still in their original adoption homes, and 80 percent were highly valued family members.

At my institution pigs, sheep, chickens, ponies, dogs, cats, rats, mice and guinea pigs have been successfully adopted out after completion of research projects. I have adopted several rats myself. They are very cute! Watching some of your favorite animals go to good homes after their hard work is quite rewarding. We have adoption forms that are almost identical to what one would fill out when adopting an animal from the humane society. I remember one instance where all 39 beagles of a study got homes after working for 2 to 3 years. It was a very, very positive experience for our entire staff!

2.9.2. Adoption by Schools

Mice are used in large numbers and are not much in demand as companion animals. But what about science classes in schools? I'm not thinking of dissections, but of "classroom pets."

Outbred rats may be a better choice than mice. They are easier to handle, respond as individuals with humans, and can readily be kept in pairs.

I agree, rats would be a much better choice for small children than mice. We have adopted Wistar rats several times now at home. They are friendly, very easy to handle—even for small children—easy to keep, and much fun to watch and interact with. I recently have kept three females together outdoors in a large rabbit enclosure. I got them as weanlings in the summer, and housed them outside when it was still warm, so they could slowly get used to colder weather. You just have to make sure that they have a warm nest. The oldest rat was with us for three years before dying in her sleep last summer. Two of the animals were once caught by a cat when my daughters forgot to close the door of the rat enclosure. It was amazing to see how these two rats survived. We found them after a few hours with bite marks and scratches, sitting in the garden of a neighbor. They were surrounded by three cats and did not try to run away. I think this strategy saved them, because the cats got bored and no longer had the incentive to attack these two unmoving critters. I treated the two survivors with antibiotics, and they recovered in no time.

My experience is also in favor of rats. They are friendlier and more robust than mice, and kids seem to bond with them better. They do learn their names and come when called. They are larger than mice and, therefore, easier to handle with little risk of being accidentally dropped. They are much less likely to bite, and they can be group-housed nicely.

Animals who have been adopted by schools often live under housing conditions that are worse than in the research lab. Many instructors/teachers have insufficient

background knowledge of the behavioral and physical needs of the animals. When ex-research animals are "adopted" by schools, it is very advisable to discuss environmental enrichment options or necessities (e.g., shelter, nesting material) with the person in charge. This will also help the children to get a better feeling for the fact that animals/pets have species-specific needs that must be met in order to keep them healthy and "happy."

2.9.3. Conclusions

Rather than killing animals who are no longer useful for research, many laboratories have started releasing animals for adoption by personnel and by private homes. Some of these adoption programs have proven to be very successful.

2.10. Individual Housing—Justifications

When is individual housing of social animals called for?

Studies in which I feel single-housing of rodents and rabbits is justified are those involving:

- cannulation,
- nutrition studies when we need to record intake/refusals,
- post-op animals after substantial surgery when the patient needs intensive nursing.

All single-caged animals *must* be housed within smell/sound of companions and, if possible, also in sight of conspecifics.

In socially housed primates, it sometimes happens that individual animals do not get along with others. It would be unrealistic to force a persistently incompatible animal to live in a social-housing situation. It is my experience with rhesus macaques that some sub-adult, 3.5-5 years old males can go through a very difficult developmental phase during which they are highly aggressive towards other males. Such animals should be caged alone, but always in visual or auditory contact with other conspecifics until they settle down, usually when they reach full sexual maturity. If there are surplus infants from breeding troops, pair-housing otherwise incompatible sub-adult males with such infants is a good alternative to single-housing. I have experienced it many times that a young male who seemingly is a monster with other males turns into a gentle, caring fellow when he gets a naturally weaned infant as a cage companion. It's amazing to witness the abrupt shift in such a male's demeanor.

Primates assigned to food intake studies are often removed from their social partners and kept alone in single-cages. This is not necessary. The daily food ration is usually distributed in the morning and mid-afternoon, and the cages are cleaned with water in the late afternoon; on this occasion, all food leftovers of the day are removed. Since the animals have no food during the night phase, there is no good

Original photo by Chris Sherwin; re-designed by Annie Reinhardt

Figure 6

Unstructured space has little value for prey animals, such as this mouse, but is likely to induce anxiety and the urge to stay close to the peripheral wall.

reason why compatible companions cannot live together during the night. They can then be separated prior to the morning food distribution with a grated/transparent panel, allowing them to keep social contact with each other during the hours when their food intake is monitored. In the evening, the panel is again removed, etc. This system helps to minimize, or perhaps even eliminate altogether, the extraneous variable stress resulting from social deprivation.

Yes, there is no good reason why paired animals cannot be put together after the last cage cleaning of the day and then separated again in the morning as you suggest. The trickier part of this schedule is to get the husbandry folks to cooperate during the weekends and take the extra time to separate and reunite the animals.

2.11. Legal Space Requirement Stipulations

Is it indicated to push for larger than minimum-size standard cages?

Individually caged animals have little or no use for extra space beyond the space required for free postural adjustments and a few normal steps/hops. Rather than "exploring" empty space, primates will climb up to a "safe" high corner of the enclosure and stay there, while rodents will show thigmotactic behavior, i.e., shunning the "unprotected" center but staying close to the walls of a barren enclosure, even if it is relatively large (Figure 6). The classical open field test (Hall and Ballachey, 1932) is based on this phenomenon: being exposed to an enclosed open area evokes anxiety. If I had to stay in a room for a long time, I certainly would prefer objects with which I can do something versus having access to another room that is completely empty. I assume that a monkey or a rat would show a similar preference to objects over more empty cage space.

The current legal minimum space and exercise stipulations of the US Animal Welfare Regulations (United States Department of Agriculture, 2002) do not make it clear that the prescribed space must be structured in a species-appropriate manner so that the confined animals are encouraged to make use of it. It is easy to demonstrate "scientifically" that animals do not exercise or play, or benefit in any manner in a relatively large but empty enclosure (Hite et al., 1977; Bayne and McCully, 1989; Hughes et al., 1989; Line et al., 1989; Line et al., 1990a; Bebak and Beck, 1993; Crockett et al., 1993; Galef and Durlach, 1993; Crockett et al., 2000). To conclude from such findings that the animals do not need more than the minimum space required for free postural adjustment would be quite misleading. Legal space requirement specifications are insufficient as long as they only prescribe quantity of space—usually based on body weight—and fail to define quality of space.

Yes, this is a crucial point. To concentrate too much on minimum space distracts from the real question, which is: What can the animal do with the space in the enclosure? More space, if not structured, will not do much to the welfare of animals in captivity. Any discussion on quantity of space needs be accompanied by a discussion on quality of space in order to be meaningful. Once you get beyond the minimum space needed by the animal for free movement and postural adjustments, the quality of space becomes much more important than the quantity of space. However, I have trouble when it comes to legislating quality of space. It would be rather impossible to write a legal document that could address each of the different species that are kept in research laboratories. I am not sure what the answer might be.

Perhaps, experts of the various species can agree on basic space *quality* provisions that should be legally mandatory, for example:

- species-appropriate elevated resting surfaces for nonhuman primates, dogs, cats and birds,
- species-appropriate shelters for rats, hamsters, guinea pigs and amphibians,
- species-appropriate burrows for gerbils,
- species-appropriate nesting material for mice,
- straw/hay for rabbits,
- species-appropriate basking areas for reptiles.

A good number of people do not need the law, telling them how to furnish the cages of the animals in their charge. I see the real problem in the fact that these people usually do not have the administrative power to implement their experience-based, often excellent ideas. Legal requirements are very important for them to give them some backing. Then there are other people who do not have the proper knowledge or do not really care. Here, professional guidelines and basic legal stipulations, defining the quality of cage space, would probably be helpful.

I am sympathetic to the difficulties of adapting inflexible regulations to current circumstances. Unfortunately, however, animal welfare often takes a back seat to other concerns, and we are left with little option for refining traditional housing practices until the inspector shows up and says we have to.

It would be a lot easier for us to improve the housing conditions for our animals if we had some legal regulations prescribing the *quality* of the enclosure space rather than just its minimum size.

2.12. Impaired Well-Being, Pain and Suffering

2.12.1. Signs of Impaired Well-Being and Pain

When you check your animals, what signs—behaviors, gestures, reactions, vocalizations—tell you that the well-being of an individual animal is impaired? We often make use of these signs spontaneously, yet they seem to be very reliable.

For most animals, the coat changes when they do not feel well. It may only be slightly "off-color," dull and "staring," rough looking with the hair clumping rather than lying sleek and glossy. Goats get a rounded face and a ridge along their backs due to the hair standing on end. Haven't observed any coat changes in sheep, but pigs will get a "fluffy" appearance when they are not okay.

Rodents, pigs, goats and sheep will take on a characteristic hunched posture when they are in pain, with their backs becoming arched and their abdomens tucked up toward their spines. I haven't observed this in rabbits. Sheep and goats will continually shift their weight from one leg to another when they are in pain, especially if the gut/abdomen is involved.

A change in idiosyncratic behaviors usually indicates that the individual animal does not feel well. For example, there may be one particular mouse who is always the first to emerge from the nest, or a certain cat who is particularly playful. When the mouse doesn't show up first or the cat is not at all playful, chances are that something is wrong with the animal.

The guinea pigs, rats, rabbits, rhesus monkeys and dogs in my charge show typical positive responses when I enter the room and approach their cages. When one of them does not move but stays quietly in a shelter or in the back of the cage, I know for sure that this animal is not feeling well and needs to be checked more thoroughly. The response to my presence is probably the most reliable indicator of an animal's state of well-being, be it a dog, a monkey, a rat, a guinea pig, a chicken or any other animal who is in my charge. This leads us back to our discussions on the human-animal relationship. It would be impossible for me to take the subject's unusual response to my entering the room and approaching the cage as a sign of impaired well-being if the animal would not have a good relationship with me, but would be scared and always hide when I approach the cage. This scenario often happens with investigators who, therefore, are dependent on animal care personnel to check the health status of the animals assigned to their projects.

Rats are very good at concealing pain and health problems. However, if one of my guys is really "ouchy," he or she may show:

- decreased social behavior,
- decreased self-grooming when a rat is caged alone,
- skin twitching especially over the back area,
- "spectacle" effect, caused by haematoporphyrin stained exudates around the eyes (Mason et al., 2004),
- decreased appetite notably for treats,
- chewing bedding material,
- not moving around,
- not responding to external disturbances,
- hunched posture. Rats who do not feel well not only sit in a hunched position but they might even walk in a hunched posture, which make their legs "look longer."

As for monkeys, I find the following signs useful indicators of an animal's impaired well-being:

- comes to front of enclosure but shows no interest in food treats (e.g., acute diarrhea, infection),
- does not come to front of cage when I approach (e.g., acute physical pain),
- does not look up when I talk encouragingly (serious health problem),
- crouches (e.g., fear, depression, physical pain),
- hides in far corner (e.g., fear),
- restless (e.g., boredom, anxiety),
- rough hair coat (e.g., chronic diarrhea),
- unkempt appearance (serious health problem).

2.12.2. Pain and Suffering

What is the difference between pain and suffering?

The whole issue of whether animals feel pain is one of logic. Pain is a subjective experience. Therefore, I can never have "proof" that you, or a monkey, mouse, cat or dog is in pain.

If we are willing to relieve discomfort only when we have "proof" that the subject—be it an animal or a human—is actually experiencing pain, we negate compassion. If you accept this inherent "feeling for another creature" you will do your best to alleviate the pain or suffering of an animal or another human being. This response is spontaneous, not a result of logical consideration.

Pain per se is a physiological, measurable, hence *objective* phenomenon. Pain is impersonal, but how it is interpreted by the subject is a subjective phenomenon that depends on the subject's relationship with the pain. Based on my own experience

I would say that animals usually do not take a painful experience personally; it's not "their" pain, just pain that needs to be alleviated and avoided. They do not resist the pain, thereby making the sensation even more intense, but respond to it in the most appropriate way possible. Humans, however, have the tendency of identifying with "their" pain, thereby turning the impersonal perception of a neutral phenomenon into a subjectively interpreted experience. The pain is now a personal problem, quasi an enemy that may trigger emotional reactions such as helplessness, self-pity, frustration, despair and worry. These emotional reactions often transform pain into suffering. Pain is unavoidable for animals and humans alike, but suffering is a choice that humans make probably much more often than animals. So it may then well be that animals usually suffer less during painful situations than we do.

This is an interesting way of looking at pain. It seems to suggest that dwelling upon pain makes the pain even more painful. It could also suggest that captive animals, unlike wild animals, have nothing that could distract them from pain, so they are at a greater risk of dwelling upon "their" pain, which would then make them suffer. Gentle and Corr's (1995) study of chickens supports this hypothesis: When chickens were placed in pairs into pens containing a deep layer of wood shavings, they showed significantly less pain-related behavioral reactions to a joint inflammation than chickens placed alone in barren pens. When tested in the barren cage, the whole of a bird's attention was occupied in trying to reduce the pain as far as possible [one-legged standing, limping, sitting]. In the more stimulating pen, the bird's attention was shifted from the pain to the social partner and the wood shavings, thereby reducing the intensity of pain that was actually experienced.

How can we define the term "suffering?"

Quite a number of authors—Balls (1994), Cockram (2004), Dawkins (1980), Fraser et al. (2000), Morton (1995), Mroczek (1994), Pollo et al. (2004), Reilly (1998), Richmond (1999), Sherwin (1998), Wemelsfelder (1993), Zimmermann (1987)—have used this term in scientific animal welfare related publications, which suggests that it does have practical value in the context of animal welfare in the research laboratory.

The lay person doesn't know what distress means, has a vague idea what stress means, but "knows" what is meant by "scientists inflict unnecessary 'suffering' on animals," because "suffering" is a term most people are very familiar with, even though they have not thought much about its actual meaning.

We cannot "objectively" measure the "subjective" experience of suffering, but this should be no hindrance for defining the term so that those who want to alleviate suffering can reason with those who inflict the suffering. Without such a definition, the animals are at the mercy of professional judgment, which is often influenced by personal interests.

1) As a **researcher**, I believe that "suffering" occurs when *an animal experiences depression, frustration, boredom or anxiety of great intensity or of long duration.*

2) As a clinical **veterinarian,** I would define "suffering" as *an involuntary exposure to a painful or injurious situation over which the subject has no control and which the subject is hindered to alleviate.*
3) As an **ethologist,** I define suffering as *the internal subjective state experienced when:*
   - *highly motivated behaviors [e.g., foraging, nest building, interacting with conspecifics] are prevented, and/or*
   - *the animal experiences stimuli that in the wild would signal impaired homeostasis, reproduction or survival.*
4) As **moderator** of this forum I summarize that suffering is *the experience of:*
   - *long-term frustration (e.g., not being able to express highly motivated behaviors),*
   - *or intense anxiety (e.g., fear of an unknown distressing situation),*
   - *or intense pain (e.g., seriously infected injury),*
   - *or intense discomfort (e.g., permanent housing on barren wire-mesh floor), with the subject having no control over the situation that causes the suffering (e.g., imprisonment).*

Even if we cannot find a consensus on the definition of "suffering," it should be possible to come up with agreeable case-by-case decisions on conditions that do or do not inflict suffering on animals in research labs. In this way, animal advocates and animal research personnel could develop common ground and dispel the myth that "biomedical research inflicts suffering on animals" but also the assertion that "biomedical research does not inflict suffering on animals." This approach is certainly better than sweeping the "unscientific" term "suffering" under the carpet, thereby making a constructive dialogue on behalf of the animals impossible. For example, if primates kept alone in barren cages, engage in stereotypical self-biting, will we not agree with animal advocates that these animals "suffer" from loneliness and boredom, even though we cannot prove it scientifically? On the other hand, animal advocates will have no good reason to argue that primates suffer when we keep them with compatible companions in cages that are equipped with high perches onto which the animals can retreat.

2.12.3. Conclusions

There are general signs—reduced alertness, lack of interest in food and enrichment gadgets, unusual coat condition, unusual response to human presence—and species-specific signs that tell you that an animal does not feel well. The response of an animal to you is probably the most reliable indicator of his or her well-being.

Not surprisingly, we were not able to reach a consensus on the definition of the term "suffering." Here is an elegant way of circumventing this dilemma:

> ***If something is known to cause suffering in humans, it should be assumed to cause suffering in animals* (Organisation for Economic Co-Operation and Development, 2000).**

I think this is a very reasonable assumption in most cases. It could certainly be treated as a starting point with any deviations requiring evidence.

2.13. Stress and Distress

The terms "stress" and "distress" are often used in the scientific literature but usually without a definition. If you use these terms, how do you define them? Are there signs that tell you that an animal is stressed or distressed?

Stress and distress are physiological and emotional responses to events:

1) An external situation (stressor) leads to stress, which implies an alteration of the subject's physiological and behavioral equilibrium (e.g., increased heart rate and fear). This kind of stress—"eustress" would probably be a more appropriate term—is not necessarily harmful, but it disturbs the subject's equilibrium, hence has the effect of a potentially data-biasing variable that needs to be accounted for in the research context. *Being approached by unfamiliar personnel is a typical stress situation*.
2) If the subject cannot adapt to the stressor, i.e., return to physiological and behavioral equilibrium, stress becomes "distress." Pathophysiological processes (e.g., chronic diseases, generalized alopecia), emotional disturbances (e.g., anxiety, frustration, depression) and/or maladaptive behaviors (e.g., self-injurious biting, hair pulling, stereotypical movements and gestures) often develop as a result of distress. *Being permanently confined in a barren cage is a typical distress situation*.

Although both "stress" and "distress" have negative connotations, distress is always bad, but stress can be both good or bad. A certain amount of stress is part of life and some mild stressors can make life a little more interesting. Introducing a new cage-mate probably causes some stress for nonhuman primates—similar to how human primates might feel when going on a first date—but, assuming the companions are compatible, this is a good stress, as it breaks up the monotony and allows the animals to express their need for social contact and social interaction. However, when stress gets out of hand, because of its intensity, frequency, or harmful nature, then that is when I say the animal is distressed. In practice, I think distress requires action to alleviate, but stress usually does not.

I consider some level of stress as normal, and, depending on the study, research conducted on animals experiencing normal levels of stress may be more biologically relevant than research conducted on animals shielded from stressors. However, it is important to be aware when stress is present, since it could affect research data, and it could develop into distress.

Stress as such is not harmful, even though it challenges the subject's physiological equilibrium. Severe stress or prolonged stress both develop into distress, when the

subject can no longer cope with the stressor and shows maladaptive responses. A stressed animal needs to be monitored carefully, while a distressed animal requires immediate assistance.

3. Maladaptive Behaviors

3.1. Stereotypical Behavior

Are stereotypical behaviors "abnormal?"

Animals kept in legal minimum-sized, unstructured enclosures very often exhibit stereotypical behaviors. Traditionally, these repetitive movement patterns without obvious goals or functions are categorized as "abnormal." A healthy animal kept in a small, barren enclosure has little choice of expressing his or her biologically inherent drive to engage in species-typical behaviors, other than pacing back and forth, running in circles, somersaulting, rocking, self-biting, bar-biting, wood-gnawing, ear-pulling, hair-pulling, eye-poking and other bizarre activity patterns (Figure 7). There is nothing really "abnormal" except the abnormally restrictive and abnormally boring housing conditions that induce the stereotyped expression of these activities. The majority of macaques who are kept in conventional barren cages exhibit stereotypical activities (Erwin and Deni, 1979; Lutz et al., 2003). These behavioral patterns thus become

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Figure 7

Sheep often engage in stereotypical gnawing when they are kept singly in a barren environment that is not appropriate for their species.

"normative" under the given circumstance. In caged mice, barbering is another example of a stereotypy that has become a normative behavior within the context of inadequate living conditions.

We tend to project abnormality onto animals rather than the people who create deficient living quarters for them. It would be fair to first focus on the husbandry conditions, study the environmental factors that lead to the development of behavioral pathologies, and then correct these factors in order to prevent behavioral pathologies in the future.

The label "abnormal" would be more befitting of the inadequate confinement condition, rather the subject's frustrated attempt to adjust.

3.2. Hair Pulling-and-Eating and Alopecia (Hair Loss)

3.2.1. Primates

Some of the cyno ladies started to lose hair shortly after arriving at our facility. There are three groups living in the same room in relatively spacious quarters that are provisioned with windows, climbing structures, visual barriers and toys. Ethological observations indicate that the groups are compatible. The ladies seem to be just fine, except for the new hair fashion they have created. Does anybody have some ideas about what to think and do regarding this phenomenon?

Compulsive hair pulling-and-eating is a common problem in single-caged and in group-housed macaques. This behavioral pathology is typically associated with localized—not generalized!—hair loss. I did ethological studies in group-housed rhesus and noticed that it was almost exclusively (378/388) partner directed and performed in 96 percent of observations by a dominant, only in 4 percent of observations by a subordinate monkey (Reinhardt et al., 1986). Based on my observations of the agonistic and affiliative interactions between group members, I came to the conclusion that hair pulling-and-eating is an ethopathology, reflecting adjustment problems to permanent confinement. It is a great challenge for social animals—including humans—to adjust to living under the same roof without possibility of taking a "vacation" from each other. Many of our group-housed rhesus and stump-tailed monkeys were almost bald. Some of them lived in a zoo, and we got many complaints from the public. I remember one particularly bad case of alopecia, *George* the ß-male of a breeding troop (Figure 8a). This gentleman showed no obvious signs of stress or distress, but we received so many complaints that we finally decided to remove him and pair him up with a juvenile male in a double cage. His hair grew back almost visibly. It was really amazing (Figure 8b). I am sure that *George was* distressed in his group, given the fact

that he had to cope with being the second ranking animal, a position that is known to be quite demanding.

I have a young male rhesus who has just started to engage in pulling and eating his own hair. I also assume it is stress-related. This monkey has been at our facility for about six months. He has not yet been used in a research project. He is very healthy, but also feisty and *very* nervous. I nicknamed him *Tarzan* because of his wild look and behavior. He shares a room with eight mature females and has visual, olfactory and auditory contact with them. Being prevented from engaging in direct sexual contact with these females must be very frustrating, and I think this is the reason why he resorts to pulling out his own hair.

I have been working with several hundred macaques over a number of years, and I have offered them all types of natural foraging and occupational enrichments, but I did not have much success in reducing, let alone eradicating, hair pulling behavior. At best, enrichment may provide a short-term distraction to deep-seated psychological maladjustment problems. Some of these problems may have their origin in a lack of basic environmental stimuli during early infant development, such as social deprivation or barren living quarters. This lack of appropriate external feedback may cause the animals to resort to self-directed strategies to get some relief of their tension. Once these critters are hard-wired it is almost impossible to change a well-entrenched behavioral pathology such as hair pulling.

Figure 8a,b

George, the ß-male of a rhesus breeding troop has lost almost all his hair (a). *George*'s fur grew back within a few weeks after he was taken out of his original group and paired up with a juvenile companion (b).

That hair pulling-and-eating is a sign of distress in nonhuman primates is supported by the fact that this behavior (trichotillomania) is associated with clinically significant distress—especially social distress—in human primates, who typically show this "mental disorder" (American Psychiatric Association, 1987) in the context of depression, frustration and boredom (Christenson and Mansueto, 1999).

3.2.2. Mice

We have some mice who are going crazy barbering and overgrooming. They have hair on their faces, but are bald from their necks to their butts with a thin strip of hair left on their abdomens. The investigator would like to try offering enrichment in an attempt to fix the problem. Currently, the animals are kept on corncob bedding with a handful of aspen bedding and a nestlet. I was thinking of adding a commercial mouse house or igloo, and maybe something additional to chew on, i.e., cardboard rolls.

Your idea of cardboard rolls is a very good one, especially in conjunction with a mouse house and shredded paper. You might also consider a more varied diet, e.g., pet mouse food, as this requires more handling and chewing.

We give our mice mini-igloos, PVC (polyvinyl chloride) tubes, egg cartons, paper towel rolls, nestlets, shredded paper towels, wood blocks, hanging plastic tubes, Kleenex boxes and running wheels, but they still barber each other! We also removed the barber in some cages. This brought some initial reduction of hair pulling, but the problem started soon again when another mouse took over the role of the barber. A "therapy" for this behavioral pathology seems to be elusive not only in primates but also in mice.

3.2.3. Rabbits and Guinea Pigs

There are hardly any published records on hair pulling-and-eating in rabbits, even though it seems that intestinal obstruction resulting from fur balls is not an uncommon cause of death in individually caged animals (Jackson, 1991; Kraus et al., 1994). How can you prevent, alleviate or eliminate this behavioral pathology?

Brummer (1975) showed many years ago that the provision of straw not only prevents the development of hair pulling-and-eating (trichophagia) in young rabbits, but also eradicates this behavior in breeding females. Rabbits are biologically adapted to process and eat fibrous food stuff, so it may well be that they resort to trichophagia as a substitute to normal food processing behavior when their diet, such as pelleted food, does not have a high enough fiber content.

Hay is probably as effective as straw in preventing this maladaptive behavior: All our single- and group-housed rabbits receive autoclaved hay on a daily basis; none of

the animals was ever observed pulling-and-eating hair. Access to hay gives them no reason to engage in this activity. This probably also applies to guinea pigs, who show a substantial reduction in hair pulling-and-eating when they are provisioned with hay ad libitum (Gerold et al., 1997).

In guinea pigs, hair pulling can also serve as a dominance gesture to make another animal move out of the way (Harper, 1976). When we used water bottles for our group-housed animals, hair pulling was a real problem, despite the fact that the animals had plenty of hay. With a bit of observation, it was discovered that this behavior occurred specifically at the water bottles, where dominant animals displaced others by pulling their hair. We consequently changed to open water dishes, and the problem disappeared.

3.2.4. Conclusions

Hair pulling-and-eating reflects maladjustment to a distressing condition in primates and mice. The inherent constraints of permanent confinement makes it very difficult to cure affected animals from this behavioral pathology. In rabbits and guinea pigs, hair pulling-and-eating is associated with a lack of fibrous foodstuff. A generous daily provision of hay or straw is probably the easiest way to prevent this behavioral disorder from developing in these two species.

3.3. Self-Injurious Biting

Self-injurious biting is a serious behavioral pathology in primates. I have videotaped rhesus macaques with the resulting impression that self-injurious biting occurs more often in singly housed than socially housed animals. Among pair-housed individuals, unfortunately, the primary trigger for self-injurious biting appears to be the mild aggressive behavior from cage mates who occasionally supplant or swat subordinate partners. In this context, self-biting does not result in visible injuries, so I will accept it for the sake of keeping pairs together. Also, there is no telling how much worse it could get if such animals were separated from their partners and transferred to single-housing.

I would argue that the development of self-injurious biting, which occurs in more than 10 percent of singly caged macaques (Jorgensen et al., 1998; Alexander and Fontenot, 2003; Novak, 2003), can be prevented if the animals are raised and naturally weaned by their mothers in compatible group settings. I was able to eradicate this behavioral pathology in seven single-caged rhesus macaques by transferring them to compatible social-housing arrangements. Some animals responded promptly to the housing modification, while others gradually stopped engaging in this stereotypy (Reinhardt, 1999). Fritz (1989) made a similar finding in chimpanzees, and subsequent

studies by Alexander and Fontenot (2003) and Line et al. (1990b) confirm them again in rhesus and long-tailed macaques, respectively.

At our facility are three adult male rhesus who had a history of SIB (self-injurious biting). The animals were treated with various drugs—diazepam, fluoxetine, guanfacine—which did alleviate but not eradicate the self-biting. Once the treatments were discontinued, the animals resorted to SIB as before. All three males self inflicted repeatedly serious laceration that required surgical care. When it was considered to euthanize these males, because the SIB could not be stopped with pharmacological therapy, we were finally given permission to pair them with other compatible companions. This "treatment" brought the self-biting to an end in all three cases. *Carl*, however, had a relapse when his companion was removed for research-assignment reasons after 14 months. Fortunately, the PI was considerate enough to drop the companion from the research protocol and allowed us to re-unite him with *Carl*, who promptly stopped again self-biting himself.

What does self-biting actually look like?

In my own experience with rhesus and stump-tailed macaques, self-biting occurs in the following two sequences of events and circumstances:

1. The subject is extremely bored, shows no signs of excitation, and repeats the same movement patterns over and over again—for example, circling, pacing or somersaulting—interjected by sham biting of specific body parts (Figure 9). This behavior often goes unnoticed because there is no visible abrasion or

Matt Rossell

Figure 9

A single-caged male rhesus macaque self-bites without actually injuring himself.

laceration, plus the subject usually does not show the behavior when there is a distraction, for example when personnel is present.

2. The subject is extremely frustrated—with high emotional arousal, e.g., shaking, intense staring, piloerection—for example, when fear-inducing personnel approach the cage, with the subject having no option of escape or attack. The animal will predictably attack specific sites of arms or legs, perhaps always the right wrist or always the left upper thigh. This typically leads to noticeable abrasion over time—first local alopecia, followed by mild inflammation—but may also result in serious wounds. Typically an animal self-inflicts lacerations of the same body part several times on different occasions, often necessitating the amputation of the repeatedly injured limb.

I remember seeing a video of a dog who would suddenly behave towards his left rear leg as if it was another dog trying to steal his food. He would growl, snarl and eventually bite one of his own legs very hard. He was an abnormal dog for sure and only one example, but I don't think self-injurious behavior is limited to primates.

When I worked in small animal veterinary practices, I saw several dogs biting their feet repeatedly. Large dogs who do not get enough exercise, can end up chewing on their hind extremities to such an extent as to expose the bone. Cats who are kept strictly indoors also engage in self-injurious biting. They attack their tails. I remember several cases that required tail amputation.

Self-injurious biting is a serious behavioral pathology that reflects gross insufficiencies in the rearing, housing and care of an animal.

4. Environmental Enrichment

4.1. Definition

What is a good working definition of the term "environmental enrichment?"

To my knowledge, the term "environmental enrichment" was originally introduced in 1991 by the US Department of Agriculture in its Animal Welfare Regulations pertaining to nonhuman primates (US Department of Agriculture, 1991). These regulations do not provide an explicit definition, but stipulate under the section "environmental enrichment" that "means of expressing non-injurious species-typical activities" must be provided.

As a **technician**, I like the following definition, which I found on the title page of the *Database on Environmental Enrichment and Refinement of Husbandry for Nonhuman Primates*:

> *Environmental enrichment is the provision of stimuli that promote the expression of species-appropriate behavioral and mental activities in an understimulating environment.*

As a **veterinarian**, I like the definition from the organization Shape of Enrichment:

> *Environmental enrichment is a process for improving or enhancing animal environments and care within the context of the inhabitants' biology and natural history. It is a dynamic process in which changes to structures and husbandry practices are made with the goal of increasing behavioral choices available to animals and drawing out their species-appropriate behaviors and abilities, thus enhancing animal welfare.*

As a **researcher**, I would define environmental enrichment as:

> *A modification that provides animals with the opportunity to do things that they seem to find enjoyable and that promote physical and mental health.*

Maureen Hargaden

AWI

Figure 10a,b
The addition of species-appropriate nesting material (a) to the standard cage of mice (b) does not really enrich the animals' environment; it merely makes it less poor.

Irrespective of its definition, I will argue that "environmental enrichment" is not a very good term for the following reasons:

1. It implies that something is added to the environment in which the animals are kept, rather than describing the environment itself.
   a) Many housing environments are so restrictive—in terms of space as well as in terms of opportunities for activity—that they will remain very inappropriate for the animals, even after the most fanciful additions.
   b) Often, any addition to the environment is understood as an enrichment, irrespective of its final outcome on the animals.
2. In the everyday use of language, "enrichment" is understood as "making richer." I could agree that, if you have primates in large enclosures with lots of climbing opportunities, different foods and nice caretakers, you may in fact

> be providing environmental "enrichment," if you give them puzzle feeders in addition. But when primates are kept in barren cages and are given puzzle feeders, that is not enriching their environment in the everyday sense of the word: it is making it less poor.

If we provide animals in otherwise boring living quarters the opportunity to engage in behaviors that occupy a major portion of their lives in the natural setting, we do not "enrich" their unnaturally barren environment, but rather provide them with basic "necessities" required for the active expression of these behaviors also in the laboratory setting. I am sure the lay person has a different understanding of the nice term "enrichment" than most of us do:

1. Do we really "enrich" a monkey cage by installing a perch and adding a social partner?
2. Do we really "enrich" a mouse cage by adding suitable nesting material (Figure 10a,b)?
3. Do we really "enrich" a cage of a rat by adding a shelter and one or several other rats?
4. Do we really "enrich" the animals' primary enclosures by allowing them to engage in foraging activities other than eating the freely available daily dry-food pellets or biscuits or chow?

I think the answer is always "no." What we do is not an act of generosity, we simply address very basic behavioral needs—and that is the very minimum that the animals deserve.

Regardless of how we define the term "environmental enrichment," it will always distract from the fact that we do not "enrich" the environment of captive animals, but provide them, at best, with opportunities to express basic behavioral needs.

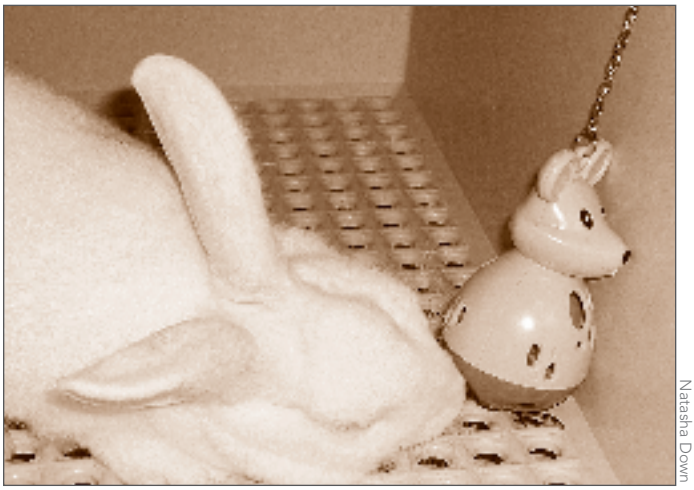
4.2. Criteria of Effectiveness

How do you evaluate the effectiveness of environmental enrichment?
The National Research Council (1998) makes it quite clear:

> *Enrichment methods that have not been subjected to empirical testing should be viewed simply as invalidated ideas, regardless of how well intended they might be. Without appropriate measurement and verification, we might do more harm than good in our efforts to improve animal conditions.*

This sounds very reasonable, but it is a given, albeit sad, reality that time, personnel and budget are limiting factors that make it very difficult, if not impossible for us to evaluate all so-called enrichment items that we give our primates and rodents.

It may not be necessary to actually evaluate all enrichment items when we differentiate between (1) biologically relevant environmental enrichment—which



Natasha Down

Figure 11

Toys have little "enrichment" value because they cannot sustain the animals' interest beyond a short-lived novelty effect.

should be mandatory—versus (2) biologically irrelevant environmental enrichment—which could be optional:

1. Biologically irrelevant environmental enrichment triggers a response that has *no* survival value for the subject, e.g., pushing a ball, manipulating or gnawing a plastic toy, looking into a mirror or TV screen, listening to radio sound. The effect of this type of enrichment needs to be evaluated by means of behavioral observations, because the animals tend to get bored by it over time. Its effectiveness is dependent on its novelty and, hence, requires regular exchange or rotation with new enrichment (Figure 11).
2. Biologically relevant environmental enrichment triggers a response that has survival value for the subject, e.g., hiding in shelter, interacting with a compatible social partner [including humans], searching for and processing food and nesting material. The effect of this type of enrichment is predetermined by its intrinsic survival value and, hence, does not lose its distracting or enriching value over time.
3. Biologically relevant enrichment is, by its very nature, effective. For example:
   - rodents do not get bored by a species-appropriate shelter;
   - mice do not lose interest in species-appropriate nesting material (Figure 12);
   - primates, dogs, cats and birds do not get bored by species-appropriate elevated resting surfaces (Figure 13);
   - amphibians do not get bored by species-appropriate basking sites;
   - animals in general do not lose interest in gadgets or other items that allow them to forage.

Biologically relevant enrichment options have been described in the literature, so there is no need to spend extra time in evaluating their effectiveness.

Pascalle van Loo

Figure 12

Animals are not likely to lose interest in biologically relevant environmental enrichment, such as nesting material for mice.

Biologically irrelevant enrichment is usually not cost effective when managing large populations of animals. This is particularly true for toys or gadgets for which the animals quickly lose interest, hence several sets of such enrichment objects are then needed to rotate them—and sanitize them at that time—on a regular basis. Therefore, taking the cost benefit ratio into account, I feel it is prudent that we put our resources and manpower into enrichment options that are relevant, producing long-term behavioral benefits to the animals.

Biologically relevant enrichment is intrinsically effective in promoting species-adequate, non-injurious behaviors, hence it does not require extra evaluation. The effectiveness of biologically irrelevant enrichment is not intrinsic and therefore has to be evaluated and reevaluated through repeated behavioral observations to assure that it promotes appropriate behavioral responses.

4.3. Feeding Enrichment

Animals in research labs usually get their daily food ration presented in a free-to-take manner, allowing little or no expression of foraging behavior, i.e., food searching, retrieving and processing. Do you try to promote more foraging behavior in the animals in your charge?

I would assume that very few animals would prefer to "forage" over sitting in front of a bowl and eating.

You may be right, but many animals will *want* to work for their food nonetheless. When you place a monkey, rat, chicken or pigeon—who have not been starving—into a cage where they have simultaneous access to a bowl with freely accessible


Viktor Reinhardt

Figure 13

A high perch provides biologically relevant environmental enrichment of which monkeys do not get bored.

food and a foraging device loaded with the same food, but requiring skillful manipulation to retrieve it, chances are high that the animal will move back and forth, eat the freely accessible food and then work for food, eat freely accessible food and then work again for food etc., spending altogether more time working for food than simply collecting and eating it (monkey: Washburn and Rumbaugh, 1992; Reinhardt, 1994a; De Rosa et al., 2003; rat: Carder and Berkowitz, 1970; Hothersall et al., 1973; chicken: Duncan and Hughes, 1972; pigeon: Neuringer, 1969). This kind of experiment demonstrates that the animals are inherently motivated to "forage," even if it implies some effort. A good compromise would perhaps be to offer them daily the opportunity to work for their standard food ration for some time, e.g., 1 hour daily feeding enrichment, then give them the bowl with freely accessible food to make sure that they get and eat enough of their ration.

The more time we can get our animals to perform species-adequate behaviors—such as retrieving their food—in boring living quarters, the less time they will spend engaged in behavioral pathologies.

4.3.1. Primates

I have given whole **watermelons** to group-housed rhesus, cynos, bonnet and stump-tailed macaques for several years without noticeable adverse effects. It would be a waste of time to cut the melons into small pieces. The monkeys first gnaw a hole into the rind and then "dig" into the soft and juicy part (Figure 14). They really like this and are kept busy until the last morsel has been eaten. They usually discard the rind, but before they do so they thoroughly remove any soft material and eat it. This usually creates quite a mess, but I don't mind cleaning it up, because the animals enjoy this type of feeding enrichment so much.

We give whole **pumpkins** to rhesus and cynos in both single- and group-housed environments. I would say that this is one of the most effective foraging "devices" we have ever given our animals. All of them spent hours processing their pumpkin!

All our group-housed rhesus receive whole **apples** on a daily basis. In order to make it more interesting for them, I place the apples into troughs that are attached to the chain-link wall of the pen 1.2 m off the floor. The animals have to climb up to the trough, reach into it and get hold of an apple, maneuver the apple up to the chain-link, press the apple towards their mouth while nibbling off pieces until it fits through the mesh of the chain-link barrier. In this way, the monkeys spend a considerable amount of time retrieving/processing apples every day (Figure 15a,b). Whole apples provide an excellent source of daily feeding enrichment also for animals who live in cages (Figure 16).

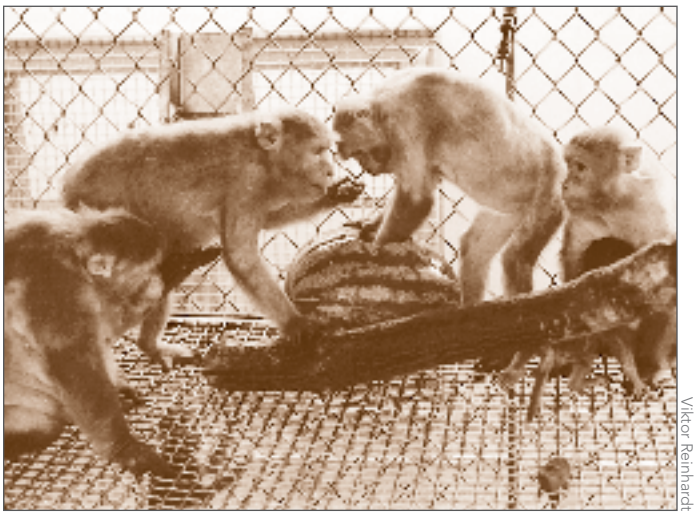
As Thanksgiving approaches, I want to give my rhesus monkeys some **cranberries,** but I wonder, do I have to worry about possible side effects for the animals?

I have fed cranberries to monkeys of several species, including rhesus macaques. They all seem to like them, and I have never noticed any negative effects.

In summer, we give our rhesus macaques **raspberries** as a special treat. They cannot get enough of them, but the juice of the berries leaves stains on the cage walls that are very difficult to remove.

I give thoroughly cleaned **sugar cane**, cut into 10 cm long segments, to our group-housed baboons. They love it! Surprisingly and fortunately, they do not leave much of a mess.

Our group-housed chimpanzees also love sugar cane, which we cut into 20-cm sections. Each subject gets about four pieces per day. The chimps chew the wedge for a long time and, doing so, give the impression that they enjoy it. Finally, the wedges are scattered all around the enclosure, which requires a bit of extra time for clean up.



Viktor Reinhardt

Figure 14

Rhesus macaques love watermelons!

Figure 15a,b

It would be a waste of time to cut apples into small pieces for rhesus macaques. The animals have all the time needed to retrieve whole apples from the food basket in which they receive their daily biscuit ration.

Sugar cane can mold easily, so it is a good idea to store it in a cool place, preferably in a refrigerator.

It would probably be fun for our macaques to get **corn on the cob**, but I am not sure if that would be a safe feeding enrichment option. I would be concerned if they ingested the cob.

I give whole corn with the husk to our pair- and group-housed rhesus and baboons. They love it, and I enjoy observing them "peel and eat," leaving a big mess

Figure 16

Whole apples provide optimal feeding enrichment for caged macaques.


Viktor Reinhardt

Figure 17

Corn on the cob allows macaques to engage in species-typical food processing behavior.

after they have finished. They gnaw the cob into little pieces that finally fall through the grid floor on the pans. I cannot say whether they actually also eat pieces of the cob, but we have never encountered any health-related problem. I don't mind cleaning up the mess; it's worth the treat!

We use corn on the cob for all our caged cynos, rhesus and vervets. The animals give the impression that they love processing and eating the corn (Figure 17). They typically pick the kernels both with their hands and their teeth. When they are done, they proceed "gnawing" on the cob. I don't know if they actually ingest pieces of it. Even if they do, we have never encountered any clinical problems.

For our rhesus macaques we fill small cardboard containers, such as glove and cereal boxes with **wood shavings mixed with food treats** and then seal them with tape really well. The monkeys have a great time opening the boxes and getting the stuff out. Some manage to get to the content without dealing with the tape. Others take their time, to first get rid of the tape, and then reach for the treats.

We also use empty plastic pop bottles, fill them with woodchips and treats, and twist the lid on tightly. Some monkeys gnaw their way directly to the treats through the plastic wall of the bottle, while others are more patient and first get the lid off. Whichever strategy they apply, they all seem to enjoy this opportunity to work for the treats. It is a little messy and you have to clean up after the feast, but it is a pretty inexpensive yet effective way of feeding enrichment.

Wood shavings in the catch pans provide an ideal substrate to foster foraging activities. On days when we change the pans—three times a week—we sprinkle sunflower seeds on the shavings. Our rhesus and squirrel monkeys then search with their fingers through the litter and pull the seeds through the floor grids, eat them or store them in their cheek pouches. Since we change the pans, rather than dump the bedding, we don't have any drainage problems in the rooms. This feeding enrichment technique doesn't require undue extra work time in our colony of approximately 130 monkeys. I'd say the benefit of being able to provide even a brief period of "natural"

foraging behavior for our caged primates is worth the little additional time it takes to put the bedding in the pans and add a handful of seeds.

With Easter upon us, I was thinking it would be fun to give my monkey friends some **hard-boiled eggs**, but I am not sure if it would be safe to have them perhaps ingest segments of the egg shells.

I have given hard-boiled eggs in shells to rhesus, cynos and baboons. Most of the animals like them, but we have a few picky eaters who refuse them. Those who like the eggs, carefully peel off the shells. I am not sure if they digest bits of them, but even if they do, it does not harm them.

We have commercial **foraging boards** for our caged rhesus and cynos. I have difficulties keeping the boards clean, especially when they have leftover peanut butter and seeds stuck in the little crevices of the Astroturf. This can be very frustrating and time consuming!

We don't use peanut butter with the foraging board, because—as you have found out yourself—it's too messy and our animals don't seem to like it all that much. We use cracked corn, white millet, whole wheat, sunflower seeds, and sweet feed—a horse feed—on a rotational schedule. As for cleaning, we just bump the boards upside down into a trash can, line them up against the wall and high pressure-hose them. Then they run through the cage washer. I'd say that 98 percent of the leftover forage base is removed this way.

I sprinkle the seeds and other small foraging items on the board, then soak it with water and freeze it. When the foraging surface of the board is frozen, the

Viktor Reinhardt

Figure 18

Converting ordinary feeder-boxes into food puzzles is an inexpensive way to foster more foraging activities in macaques.

animals spend a lot more time picking the seeds and crunching on the ice. Our monkeys are having a great time with this kind of feeding enrichment.

Our pair-housed and group-housed rhesus macaques retrieve their daily biscuit ration through the **mesh ceiling** of their cages and pens (Reinhardt, 1992a; Reinhardt, 1993a). This allows them to engage in skillful foraging activities that keep them quite busy. This kind of feeding enrichment is very effective, although it does not cost anything. You simply throw the biscuits up on the top of the cages or pens rather than distribute them in the feeder-boxes.

I have converted the ordinary feeder-boxes of our caged rhesus and stump-tailed macaques into **food puzzles**, by remounting them away from the access holes directly onto the front mesh walls of their cages (Reinhardt, 1993b; Reinhardt, 1993c). Rather than collecting freely accessible biscuits, the animals now have to use skillful foraging techniques to retrieve their daily biscuit ration (Figure 18).

Do your animals keep normal body weights when they have to work for their daily food ration?

Yes, working for food, rather than having free access to it has no noticeable effect on the animals' body weight maintenance (Reinhardt, 1993a,b,c,d).

4.3.2. Mice and Rats

Corncob bedding provides a great foraging enrichment substrate, because it invariably has small pieces of corn hidden in it. Every time we change the cages, the mice scurry around searching for the corn.

We were scattering sunflower seeds on the paper-based bedding of our mice until a researcher, who agreed at first, complained that the body weights of the mice were yo-yoing. This was the end of this foraging enrichment attempt. The increased variation in body weight was caused by the fact that the mice anticipated the sunflower seeds eagerly but did not touch their normal food pellets!

Doesn't that tell us something about the palatability of the pellets?!

Yes, but it also tells us something about the animals' strong motivation to forage!

We buy cracked corn and wild birdseed mix, add popcorn, a few sunflower seeds in the shell, and occasionally some dry cereal or fruit-flavored bird treats. Toward the end of the day, I scatter a small scoop of the mix around the cages of our rats, and then add a little portion on top of the pellets in the hopper so that a few treats will trickle down here and there when the animals retrieve the chow. The daily provision of this mix keeps the rats busy for quite a while, and they really seem to enjoy it.

Wrightson and Dickson (1999) designed a feeding arrangement for rats that helped to prevent obesity, by making the animals work for the retrieval of their standard food ration. Unintentionally, these authors came up with a very simple feeding enrichment option: Group-housed rats were induced to work for their food

by soldering metal plates over their food hoppers, so that only 3 percent of the original area remained available. The animals fed for longer periods and rested less during the night. No changes were observed in the rats' social hierarchy and there were no increases in fighting with restricted hoppers, as up to three rats could feed at a time. It was felt that this method of food restriction was preferable to giving less food to avoid obesity. Rather than rapidly eating a reduced ration and feeling hungry for long periods, the rats worked harder for their food, which enabled them to burn more calories and eat throughout the day. This reduced the incidence of obesity while encouraging the animals to engage in more food-related activities.

We keep jars of sunflower seeds for mice, and jars of whole peanuts, cereals and dried fruits—especially apples—for rats on the counters in the animal rooms, so that attending personnel can distribute treats whenever they are inclined to do so. These regular visits enriched the daily routine not only of the animals but also of the personnel. At the same time, they foster a positive human-animal relationship.

4.3.3. Guinea Pigs and Rabbits

Our guinea pigs and rabbits get a wide variety of fresh produce—we do not chop the veggies—including dandelion greens and curly kale. The animals seem to enjoy processing and eating this natural food without any adverse effects. There is only one investigator concerned about pesticides, so the food must be scrubbed, peeled or grown organically. Besides that, none of our investigators has a problem with their animals receiving fresh produce as a means of feeding enrichment.

4.3.4. Cats

Our cats receive their pellet diet in simple food puzzles consisting of recycled cardboard rolls of paper towels. A few pieces of cork are glued into each roll, making it more difficult for the cats to retrieve their food. The animals don't get tired of "stalking" their "prey," waiting for the prey to emerge, and retrieving it with dexterous manipulation from the "burrow" (Figure 19).

We are using a similar device that consists of a plastic ball with a few holes just large enough so that the cats can maneuver food pellets through them. They not only engage in cat-typical foraging activities, but also play with these balls. It is definitely more fun for the staff to watch the cats playing with these enrichment gadgets instead of sleeping. We have all noticed that our cats have become easier to handle in their enclosure, as well as easier to catch, since we have introduced these balls. My own cat has also had one for three years. She made it clear right from the beginning that she prefers having her daily food ration distributed in the ball rather than in the boring food bowl. Obviously, she likes to "work" for the retrieval of

Annie Reinhardt

Figure 19

This is a simple but very effective foraging device loaded with the standard food ration for cats.

her food, similar to a free cat who probably prefers to spend time hunting for a live mouse rather than pick up a dead mouse.

4.3.5. Sheep

Busterballs filled with grain work well as feeding enrichment gadgets for our post-surgical sheep. They spend lots of time head-butting and kicking the balls around the pen in order to retrieve the grain (Figure 20).

4.3.6. Objections by Investigators

Do investigators accept the feeding of supplemental fresh produce or treats as part of your environmental enrichment program?

Investigators regularly object to the introduction of enrichment—whether it is food or toys—because they fear for the comparability of their studies with previous work or with the work of others who do not provide enrichment. They insist on keeping their animals under "standardized," albeit species-inadequate environments under the pretext that environmental variables need to be controlled to make the study a truly "scientifically valid" study, yet they tacitly overlook basic variables such as the investigator himself, new caging design, cage location, new or renovated animal holding facility, etc. Using a double standard when it comes to extraneous variables may be convenient, but it is not at all scientific.

Tamara Godbey

Figure 20

Busterballs filled with grain keep sheep quite busy retrieving the food treat.

4.3.7. Conclusions

Feeding enrichment is a practical option for animals kept in research laboratories. The regular provision of thoroughly cleaned, whole fruits and vegetables and of seeds scattered on woodchips or corncob bedding is probably the easiest yet most effective way to promote species-typical food searching and food processing activities in primates, rodents and rabbits. For cats, standard dry food can easily be presented in such a way that the animals can engage in cat-typical hunting-related behaviors.

4.4. Coconuts

Do coconuts provide suitable and safe environmental enrichment?

I am involved in a project in which we are examining various enrichments for mice. One of these is coconut shells that the mice seem to enjoy immensely. They climb on them, use them as olfactory look-outs—rear on their hind legs and sniff the air—use them as shelters, and chew, chew, chew, chew on them! Often, the mice chew the coconuts from the inside, so when we pick the shell up a week later, it is paper-thin!

Rhesus don't care much about coconuts, but stump-tailed macaques are fascinated by them and do not get tired "working" on them until the last morsel has disappeared in the drop pan. It never occurred that one of the monkeys somehow became injured while processing a nut.

I give whole coconuts to our individually caged cynos. More than anything, they like them for grooming purposes. It gives them something else to do besides bite themselves. I also had a female who carried her coconut around as if it was a baby, constantly clutching it to her chest, and lip smacking to it, grooming it, etc. She was a chronic alopecia case. The coconut alleviated some—unfortunately not all—of her stereotypical hair pulling behavior.

Whole coconuts seem to provide effective and safe environmental enrichment for macaques and mice, and presumably for other rodents as well.

4.5. Mirrors

Can anyone share first-hand experiences on the usefulness of mirrors as enrichment objects?

All of our single-housed long-tailed **macaques** have mirrors mounted on swivels that are attached to the outside of their cages, low enough so that an animal can chose to either bend down and intentionally look into the mirror or to make no extra effort, hence not be confronted—bothered?—by the mirror reflection. Our monkeys use their mirrors frequently.

We hang stainless steel mirrors right into the cages of our macaques. Some monkeys will cling to them and look at them for long periods ot time, often lip smacking or making other facial gestures, while others will threaten their own reflection and bang the mirror onto the side of the cage. There are a few animals who "attack" their own reflection in the bottom of the stainless steel cup when it is empty. It's quite hilarious!

Most monkeys use their mirrors to look around the room at other monkeys or at people, whom they could not normally see. I assume that the animals feel more at ease when they can avoid direct eye contact with personnel and other monkeys, yet can observe them without being noticed. It's fascinating to watch them moving the mirror in the right position so that they can look at a person, who is not in their field of vision (Figure 21).

Our rhesus love mirrors too. They like to check us out by looking at us through the mirror. I guess they don't feel so threatened when they can look at us without being seen. They also like to check out the room, by looking at the reflections in the mirror. We have one male who never looks at people directly, but holds up a polished stainless steel mirror to watch people who have just entered the room. Of course, we named him *Mirror Man*.

We have found an acrylic sheet mirror that we can cut into different-sized pieces. Some get hung on the walls, using double sided tape, while other pieces get hung right inside the enclosures, using zip ties. We also cut small pieces and give these directly to the primates. Our rhesus macaques often combine the wall and hand mirrors to

Richard Lynch

Figure 21

A cyno male uses a mirror to watch a person who is not in his direct field of vision.

get extra viewing advantage! It's really fun to watch them. The acrylic leaves no sharp edges when it breaks; this means it is safe for the animals. We never encountered a problem.

It has been shown in rhesus macaques that mirrors serve not only as enrichment gadgets, but that they can also promote social facilitation, with the mirror reflection of another animal playing with a toy triggering the interest to do the same (Baker, 2000). This is an elegant way of enhancing the novelty effect of enrichment objects, at least in primates.

Our singly housed **baboons** get the most enjoyment from their mirrors, while pair- and group-housed animals show little interest in them. We place the mirrors on the outside of the cages of our single-caged baboons, leave the mirrors only for a few hours at a time and replace them after a few days. This seems to work nicely: The animals' interest in the "new" mirror is always very strong, gradually declines and is hardly noticeable at the end of the day, when we take the "old" mirror away. Often the baboons will lip smack the mirrors or use them to look around the room. One boy was recently seen presenting to the mirror! I think that mirrors offer great enrichment to the animals.

I have a male olive baboon in my charge who regularly sits for long periods at a time looking at himself in a mirror. He is housed with two females but appears to prefer looking at his own mirror reflection versus the nice tumescent females hovering around him! He also uses his mirror to see reflections of what is going on behind him, sitting diagonally with his back facing the main traffic area for techs, as if he was spying on us! I do believe he is entertaining himself quite a bit with the mirror.

We use stainless steel mirrors for our vervets who, just like the macaques, use them to look at either themselves or at other monkeys. Harris and Edwards (2004) studied singly housed animals and found that individuals contacted mirrors, hung on one side of their cages with a 18 cm long chain, about 5 percent of the time. Habituation did not appear to occur even a year after the mirrors were introduced.

I have videotaped singly caged **rabbits** who had constant access to a mirror

mounted on the inside of their cage. Neither does nor bucks were attracted by their mirrors, even though they seemed to perceive their own reflection in the mirror as a social counterpart (Figure 22). Jones and Phillips (2005) found that single-caged rabbits do show initial interest in mirrors, but that this novelty effect wears off very quickly.

Sherwin (2004) concluded from a preference test study that a mirror can be aversive to singly housed **mice,** especially during feeding. It might be that for mice—who use olfaction as their primary sensory modality—the "confusion" of seeing another mouse with no smell is frightening. Obviously, a mirror is not a suitable enrichment gadget for them.

Sheep, who are housed individually for research-related reasons, typically become extremely skittish and vocal for long periods of time. McLean and Swanson (2004) mounted a large mirror on one wall of single-housing units. Isolated sheep stood close to and nudged the mirror image without showing any signs of agitation. The risk of injury was eliminated, as the sheep no longer tried to jump or escape the enclosure. Parrott et al. (1988) also emphasize that isolated animals show considerable interest in their mirror reflection, and that physiological stress reactions to social isolation are lower in sheep with a mirror versus without a mirror. Piller et al. (1999) made a similar observation in cattle and concluded that the mirror-image reflection seems to buffer isolation stress.

Mirrors provide useful environmental enrichment for primates. The literature suggests that mirrors may help to buffer isolation stress in some species.

Joanne Edgar

Figure 22

Rabbits do respond to mirrors at first, but they quickly lose interest in them.

4.6. Music

Does sound or music have any environmental enrichment value for animals in research labs, other than keeping the attending personnel in a good mood?

Several people at our facility request to have radios in their rodent colony rooms to act as a sound buffer. If that's a good reason, my preference would be to have the radio set to static or have an actual white noise generator. I've found that some technicians and care staff play the radios so loudly, you can hear them outside the animal rooms, sometimes even in adjoining rooms. I am lucky and can just leave when the noise gets too much on my nerves. The animals have no choice but listening to this cacophony, probably not a situation that is animal welfare-conducive.

We have had an ongoing problem with people playing radios in the animal rooms at excessive volumes, which could drive me—and probably also the caged monkeys—crazy! There was no way to get people to change their habits voluntarily, so we had to make it a rule that no radios are allowed in the animal rooms and in the corridors. What a difference it made!

We found a compromise: Rather than playing radios, we play CDs with classical music at a background volume that cannot be changed by the attending personnel.

It's probably not only the volume but also the quality of music that can affect animals differently. Our monkeys used to be exposed to rock music. We then switched to classical music, and I have the impression that the animals are now calmer and much easier to work with. Brent and Weaver (1996) noted a decrease in heart rate in baboons, Howell et al. (2002) an increase in social grooming and fewer aggressive interactions in chimpanzees when the animals were exposed to classical music.

Primates, being diurnal animals, may enjoy listening to certain types of music during the daylight hours, but rabbits and rodents are nocturnal animals who want to sleep during the day. I would not think music is beneficial for them, even if they don't show any specific reactions to the music. I don't have any experience with music in rabbit rooms here at work, but I do have a pet rabbit who very clearly prefers *not* to be in the same room as loud or fast-tempo music. He will simply leave the room.

We have a radio playing in all our rooms including those of the rabbits. The radios are left on round the clock with the aim of providing a constant noise environment that may help the animals to better cope with disturbances for example, not to be startled if someone enters their room. The radio-created noise in the animal rooms is kept so low that you cannot hear it in the corridors.

Background music can have a calming effect on caged primates. We do not really know if being forced to listen to loud music of the personnel's liking is also to the animals' liking. If it is not to their liking, chances are that they feel distressed. This probably holds true, for rodents and rabbits, who are biologically adapted to sleep during the day.

4.7. Windows

How useful are windows for environmental enrichment?

Windows are particularly attractive for **cats** who, biologically, are intensely motivated to keep visual control of their immediate living environment. On the basis of caregivers' perception, most cats look out of windows for at least five hours a day (Figure 23; Shyan-Norwalt, 2002).

We expose our **squirrel monkeys** to natural daylight via big windows during the summer. This is supplemented with artificial light in late fall and early spring, when the days are short, and throughout the winter. Some of our squirrel monkeys will lie as close to the window as possible and let the sun rays dance on their belly.

I've seen the same behavior in our **marmosets**. As soon as the sunlight hits the window, the animals stop what they are doing, run over to the window ledge, and start stretching out and basking in the sunrays. There is no doubt in my mind that exposure to natural light, especially sunlight, is highly appreciated by the animals.

Geoff Loveridge

Figure 23

An exterior window provides optimal environmental enrichment for cats.

All our rhesus **macaques** have access to one-way glass exterior windows mounted high above ground level. I very often see the animals gather up, attentively gazing out of the windows towards the source of some noise, at caretakers, activities in the garden and birds. One would think that exposure to daylight and the natural diurnal rhythm couldn't be anything else but a good thing for these animals.

I remember visiting a facility that had constructed a playroom for male cynos with a window facing outside. The attending personnel told me that the animals spend more than half of the day, during which they are released in this room once a week, on the shelf looking out of the window, ignoring all the other environmental enrichment gadgets, including toys and mirrors, most of the time (Figure 24; Lynch and Baker, 2000).

This playroom with a window is a great idea! Our facility is in a basement with no windows, just artificial light, which I think is a bummer. Our monkeys never experience natural light or a vista of something more natural beyond the walls. I am sure they would also love an outdoor view. To me it always seems a depressing ambiance in which our animals are forced to exist behind bars. My office is in the animal quarters and consequently has no window, but I have the freedom to leave that "cell." I am sure that lack of natural light does affect nonhuman primates in a similar manner as it does human primates, who can get SAD (seasonal affective disorder) during the winter when the possible exposure to sunlight is decreased by many hours. The great majority of caged nonhuman primates are *never* exposed to natural light, let alone sunlight.

I have often also thought about this, wondering how nice it would be for our monkeys, if we could put some skylights in their room. I am sure my facility would not go for it!

External windows provide optimal environmental enrichment for diurnal animals.

Richard Lynch

Figure 24

Exterior windows can provide macaques with species-adequate distraction in which they do not lose interest over time.

4.8. Toys

Blankets, stuffed dolls and teddy bears are items that are highly valued by human toddlers, and I would guess also by **macaques**.

Many of our rhesus and cynos either shred blankets or "stuffies," or they are deathly afraid of them. We had a male rhesus who was donated to us for retirement. *Ozzie* came with a "blankie" that he loved dearly. He groomed it and carried it around. He was extremely protective of *Blankie* and only gave it up after he was successfully integrated into a group.

There is a rhesus male in our facility, who is very attached to a "purple stuffed monkey." He grooms his buddy daily, becomes fiercely protective when the stuffed monkey is removed for cleaning, and even tries to take it along to the restraint chair. *Patch* never attempts to rip the stuffed animal apart, but acts as if it is his social partner. It's so funny! The technician responsible for his care has to hunt for other "purple stuffed animals" in order to replace them when *Patch* has worn them out. Brown and yellow stuffies will not do, they have to be purple!

We use an assortment of kong toys for our pair-housed rhesus **macaques**. I find that they do not pay much attention to them, unless I have stuffed them with some food treats or filled them with frozen juice. Once the contents have been consumed, the toys are pretty much ignored. The little interest they show in plain kong toys does not differ with the little interest they have for their other commercial toys. Crockett et al. (1989) made a similar observation in single-caged long-tailed macaques.

It is my experience with macaques that the animals show no habituation to destructible, yet biologically irrelevant enrichment such as cardboard boxes, telephone directories and gnawing sticks, but quickly lose interest in indestructible enrichment such as hard rubber toys and nylon balls.

Some very simple toys may become quite attractive, depending on how the animals may actually use of them. When I worked with baboons, we had several males who never tired of their metal cans. Part of the appeal was that, without fail, they would bang the cans very loudly right when you least expected it. You'd be working and the room would be very quiet with just the occasional "coo/whistle" and then suddenly "bang, bang, bang." We'd always jump, and I think it was our reaction—not the cans—that brought the most entertainment.

Dogs quickly lose interest in any toys, unless a human caregiver entices them to play (Figure 25). There are a few dogs who enjoy chewing on them for a while, but the majority don't. I have also suspended a few nylabones on chains. Some dogs chew on them quite a bit, but most are not interested, and after a few days ignore them.

It is sometimes recommended to exchange toys on a regular basis (rotation) to

Animal Welfare Institute

Figure 25

Dogs tend to lose interest in commercial toys, unless a person entices them to play.

recreate novelty effects. How practical is this recommendation in your situation?

I am working with several hundred **rats,** and I rotate their toys. Novelty "returns" after an item has been taken away for a few weeks. Rotating toys to provide novelty effects isn't really that much of an effort. Storing and sorting them is the harder part for us.

Rotating toys for our caged **rabbits** is actually very easy. If it's noticed that a rabbit is not interested or has lost interest in a particular toy, we simply exchange it with another toy during the morning health check. Otherwise, all enrichment objects are rotated on a routine basis when racks are changed.

We have approximately 500 caged **macaques**. Their toys are rotated every two weeks. This is practicable.

Destructible toys are usually more attractive and of longer lasting interest for animals in research labs than indestructible toys. For dogs, toys become interesting when personnel entice them to play. Rotation of toys every two weeks is practicable even when this involves a large number of animals.

4.9. Paper-Based Items

Does anyone offer paper or cardboard boxes as enrichment to the animals in their charge?

I use brown paper bags as "foraging bags" for our rhesus **macaques**. I mix a bunch of cut up fruits, seeds, veggies, and peanuts and wrap them up in a bag. The monkeys rip the bag open and dive in! They seem to enjoy it. Most of them will just eat what's inside, and some will also go for strips of the paper bag. I had a couple of

husbandry complaints because the paper would sometimes stick to the cage walls and had to be removed by hand during the hose-cleaning of the cages.

You can avoid this problem by replacing the bags with cardboard boxes. I use empty glove boxes, fill them with shavings mixed with dried fruit and other treats. The monkeys absolutely love them! When they see me coming into the room with treat boxes, they get all excited. Within seconds of receiving the boxes, the monkeys have pulled everything out and proceed eating the treats, leaving the boxes alone for a while. By the next morning, the boxes are completely shredded. Cleaning up the mess is not a big deal for me; it's worth it since the animals have such a great time with these enrichment gadgets.

My rhesus and cynos get paper towel rolls and old phone books. While the cynos will often chew the paper material, the rhesus typically shred it. Animal care staff don't really like this kind of environmental enrichment, because the paper gets stuck to the bottom of the pans. Since the animals really like it, we struck a compromise, offering them paper enrichment not daily, but at least two times a month.

I gave group-housed rhesus macaques (16 animals) one cardboard box once a week and made observations after a habituation period of eight weeks. During the first 120 minutes after cardboard distribution, individuals spent on average 78 minutes tearing the box apart and chewing pieces of it (Figure 26; Beirise and Reinhardt, 1992). At the end of the 2-hour observation sessions, the cardboard box was shredded into pieces that were so small that they did not cause problems with the routine cleaning; no clogged drains! The cardboard box then became a standard enrichment item for group-housed animals.

We recycle cardboard boxes and big paper bags in our **rabbit** playpen. Most animals use the boxes as a look-out post, but some will scratch at them, tip them over and use them as an alcove. Typically, the cardboard boxes are vigorously batted around the cage, so they don't last long enough to get too dirty. The bags make great "tunnels"

Viktor Reinhardt

Figure 26

Recycled cardboard boxes do not cost anything, but rhesus macaques appreciate them as a source of entertainment.

Viktor Reinhardt

Viktor Reinhardt

Figure 27a,b

Rhesus macaques do not lose interest in their gnawing sticks. They like to gnaw at the sticks (a), manipulate them (b) and drag them around.

that the rabbits will run in and out of. Some animals chew on the outer part of the bags, but for the most part leave them intact but well-stomped. The great thing is that when the rabbits are done with a box or a bag, you simply throw it out and replace it with a new one! It's a very inexpensive way of giving rabbits something to do in an otherwise boring environment.

We also have tried shredded paper, but our rabbits don't seem to enjoy it as much as the cardboard boxes and the paper bags. The rabbits turn shredded paper quickly into a stomped, wet soggy mass. This is not a good idea for enrichment!

I often observe some of my techs "feeding" strips of soft paper towels through the cage fronts to our **rats**, who then enthusiastically chase whoever is in possession of the strip and try to grab bits of it. The one who has the strip tries to sit on it or wrap it around her body, and you finally end up with a pile of rats and lots of smaller pieces of paper. Once the paper is torn up, the game is over. We have not seen any injuries during these games nor any signs of overt aggression. I am not sure who enjoys it more—humans or rats!

Cardboard boxes offer inexpensive and practical yet effective environmental enrichment for primates and rabbits. Rats enjoy playing with paper strips.

4.10. Wooden Objects

I give our single-caged baboons 20 cm long gnawing sticks made of pecan branches. They *love* them! It takes one to two weeks for a stick to be whittled down to about half of its size.

Gnawing sticks cut from dead red oak branches provide inexpensive enrichment for macaques (Figure 27a,b). The animals do not get bored by these sticks which, due to gradual wear and progressive dehydration, keep changing their texture and configuration, thereby retaining novelty (Reinhardt, 1997).

We use aspen sticks for all our rodents and rabbits. They are soft enough for a good "bite." These sticks are used heavily, which suggests that the animals like them. The sticks can be sterilized for use behind barriers. Normally, they are changed every two or more weeks, depending on how soiled they are and how much is left of them. From my experience, gnawing sticks do not lose their attractiveness over time, probably because they allow rodents to fulfill their inherent drive to engage in gnawing.

Properly sized and properly cleaned/replaced wooden objects provide inexpensive but effective environmental enrichment for rodents, rabbits and macaques.

4.11. Running Wheels

Is there any evidence that access to running wheels can prevent the development or decrease the incidence of behavioral disorders—such as barbering?

Gebhardt-Henrich et al. (2005) found that single-caged hamsters show significantly less stereotypical bar-mouthing when they have access to running wheels. Similar findings have not been published for mice and rats.

Do mice compete over access to one running wheel?

The answer is definitively "No." We have often seen several mice on

Maureen Hargaden

Figure 28

Properly sized and designed running wheels provide effective environmental enrichment for rodents.

one running wheel, and never witnessed any antagonism related to the wheel. It's not uncommon to have two mice running in the wheel and one or two, in addition, running on the top of the wheel (Figure 28). It is quite a sight!

Do "old" rodents have any use for running wheels?

Running wheels are great for young and adult animals who have the energy to exercise. Aged mice may sit in a wheel, but they are unlikely to run in it. I remember a study in which "aged" rats—24 months old—were tested on running wheels. These animals had hardly any use for the wheels. The researcher tested two separate groups of aged rats, and neither of them was interested in the wheels. For old rodents, an object for gnawing and manipulation is a better enrichment idea than a running wheel.

Running wheels provide suitable enrichment for rodents.

4.12. Burrows

I use 10 cm deep carfresh bedding, along with cardboard tubes and nestlets, in regular mouse cages. The mice build amazing nests and dig tunnels in this paper-based substrate. It is quite a revelation to see laboratory mice burrow in substrate. I always have a broad grin on my face when watching mice dig so furiously that they flick the substrate out of the cages and all over the place—a technician's nightmare! The mice build the tunnels along the sides of the cage—touching the sides (thigmotaxis) seems to be reassuring to them—so you can see them running about and behaving in very different ways underground. It's fascinating to watch!

Mice readily work to gain access to a suitable burrowing susbstrate, and they are more motivated to burrow in it than run through a tunnel (Sherwin et al., 2004). This suggests that burrowing constitutes a "behavior need" for them that is not satisfied by an already prefabricated burrow.

We give our rodents lots of shredded paper, straw and/or hay that they tunnel through and use for nesting. However, to check every animal on a daily basis can be a challenge with mice. To take the lids off the cages and search for mice amongst the nesting/burrowing substrate is relatively time-consuming and also probably causes considerable stress for the mice. This is less of a problem with rats who, unless sick, nearly always come to the front of the cages—even if this implies leaving a shelter—to see who is approaching their cage.

Rats will use about anything that can cover them, even if it's not really suitable. I have videotaped a rat who tried hard to dig into and burrow under a handful of wood-wool to become invisible to my presence. It is very important to realize that the domestication of rats has not eliminated their inheritance of being a prey animal. Their sense of security is very much dependent on being able to disappear from sight

quickly, either by seeking cover or ducking under in a burrow. Unlike mice, rats will readily accept a prefabricated burrow.

The need to dig a burrow is probably not as strong in rats as it seems to be in mice. In sharp contrast to rats—who are very curious—mice are reluctant to leave their burrow in order to be checked, unless they know you very well and have good reason to trust you.

4.13. Gerbil Idiosyncrasies

I recently adopted two female gerbils who were used to test a new ventilated housing rack. I have them in a snake aquarium—there's no snake in there!—and initially had them housed on aspen chips. Several months ago, I changed their environment completely. I replaced the aspen chips with a 10 cm layer of ground walnut shell, into which I buried a PVC pipe with bends and elbows so that three openings were positioned above the shell. Here are my questions:

1. The gerbils seem intent on burying their food dish and they seem to do it deliberately: They'll jump in the bowl, sniff about, and then jump out and shovel walnut shell into the food bowl, then repeat the entire procedure. **Is there a better way of providing their feed other than spreading it across the cage?**
2. The gerbils move their nesting paper every few weeks to different corners of the tank. **Is this normal or does it indicate that something in the environment is stressing them?**
3. **How much space do gerbils need?** When they were on aspen chips, the tank seemed more than spacious, judging by how much of the space they actually used. Now that they have a digging substrate, they would probably be happy with an enclosure that took over the entire room.

Since gerbils are proficient diggers, I always give them at least 30 cm of substrate, consisting of wood chips, hay, straw and twigs. I also add branches and cardboard boxes to stabilize this substrate. Stable burrow systems can only be constructed if the enclosure is big enough. For your two females, I would recommend living quarters with a floor space of at least 100 x 50 cm and a height of at least 50 cm—the bigger the better.

If the substrate is stable enough, the gerbils will not need an artificial burrow system made of pipes, but they will prefer to construct their own burrow. The burrow will be constantly "under construction" and change practically every day.

Occasionally moving the nesting paper to different places is a biological normal adaptation to the fact that the nest might become infested with parasites, if the animals contact it for too long a time period. I have studied gerbils kept in moist

Pascalle van Loo

Figure 29
When given the choice C57Bl/6 mice will build their own nest with appropriate material—here paper tissues—and sleep in it rather than make use of a red plastic mouse house.

sand-filled tanks that were designed in such a way that I could observe the animals almost everywhere within their burrow system. It turned out that gerbil families change their nest site every three to five days!

The strong urge to bury the food that is not stored in food chambers is also a biologically normal behavior, as food competitors—in the natural environment, especially the steppe vole—might steal it. Even if you scatter the food all over the substrate, the gerbils will first store some in food chamber-like places and then bury the rest. There is nothing you can do about it.

4.14. Shelter and Nesting Material

What kind of shelters and nesting materials work best for rodents and rabbits?

4.14.1. Mice

4.14.1.1. Indestructible Material

We use the commercial plastic **mouse house** in combination with cotton nestlets. The mice use these shelters regularly. Some investigators noticed a better breeding performance when their mice had access to a mouse house plus a nestlet. As a result of this, most of our mice have now a mouse house along with a nestlet.

My experience with the mouse house is not so favorable. I have noticed that, in a cage furnished both with the house and with paper tissues, mice will typically drag the tissues to a suitable location *away* from the house, build a nest and sleep in their own nest rather than in the house (Figure 29).

I have made a similar observation. Some of our females with pups just don't like this sturdy "mouse house." I would place a mother with her litter in a house, but she would soon move the whole litter out. I repeated this game several times, always with the same result. Some mothers simply refused to stay in these houses and preferred building their nests *outside* with paper tissues. It's not really surprising that some—perhaps most—mice prefer to construct their own nests according to their mice-specific microclimatic needs, and sleep in them instead of a prefabricated structure.

We have a group of mice who, without apparent reason, showed a decline in breeding performance. After we placed plastic **mouse igloos** and nestlets in their cages, these mice returned to their normal breeding performance. I have seen some of them take their nestlet into the igloo—where they probably built their nest—and keep their pups under the igloo. Possibly, the mice feel more secluded in the relatively small igloo, while the much bigger mouse house may feel too open for them. The igloo is also less heavy than the big mouse house, and the mice can push it around, adjusting the entrances/exits exactly the way they want them to be.

4.14.1.2. Destructible Material

We have tested cotton **nestlets** in several strains and found that: MF1 nudes shred them and build nests, and ordinary MF1 and Balb/c mice seem to ignore them; the same is true for C3H mice. Some C57Bl/6 mice shred them or sit on them, while others also ignore them.

With the strains that don't use the nestlets, it's almost as if the mice don't recognize them as nesting material. It might help if you started them off, but it would be quite fiddly, and I don't think the advantages over shredded paper are sufficiently clear to warrant the extra labor, especially when you have several thousand cages to deal with. We have stopped using the nestlets, as all strains of mice that we work with seem to be "happy" with shredded paper. The additional advantage of shredded paper is that it costs nothing.

When given a choice between a **paper-based** and a plastic nestbox, mice *always* choose the paper box. Usually they sleep inside this box and, when given nesting material, they drag it into the box and build a nest (Van Loo et al., 2005). When no extra nesting material is available, they will shred the paper box and use the shredded material to build their own nest and sleep in it.

We use hardpaper igloos. The mice climb on them, chew holes in the walls, and mark them with urine, thereby giving a personal touch to their homes. When we move these urine-impregnated igloos during the cage cleaning process to the new cage, the mice are much less restless and aggressive among each other. They probably feel "at home," as it literally smells like home.

I think you are right. We give our mice paper-based nest boxes that we also move

along with the animals into fresh cages. The repeated transfer of the soiled nest boxes and the scent marks adhering to them probably accounts for the fact that we also see hardly any fighting in strains considered to be conspicuously aggressive. Over the six years that we have been using paper-based nest boxes, we have encountered no ill effects on the mice's health status.

Our mice get cardboard boxes brought in from home by animal care staff. We first autoclave these items before placing them into the cages. The animals seem to enjoy the boxes, and we like to think we are being "green" by not wasting paper. It often raises a smile to see a gang of rats or mice using an empty cat food box as a house. Who said animal techs don't have a sense of humor! We also use egg cartons, which autoclave very well. The mice explore the little "huts" and quickly turn the cartons into shredded pieces that make a good bedding and can be turned into nests.

4.14.2. Rats

In contrast to mice, rats have a strong preference for solid shelters. They have little use for nesting material unless it comes with a secluded shelter in which the nest can be built. Both female and male rats will move suitable substrate, such as straw, into a shelter and build well-formed nests even when they have never before been exposed to nesting material (Figure 30; Jegstrup et al., 2005). Rats will rest in a shelter during the light period, and climb on it and spend much of the time resting in that elevated position during the light period. Almost any type of solid shelter will do for them, but they seem to have a particular preference for opaque boxes with two or more small entrance holes (Patterson-Kane, 2003).

Inger Jegstrup

Figure 30

Rats will move straw into a shelter and build well-formed nests even when they have never before been exposed to nesting material.

Maureen Hargaden

Figure 31

Round plastic pipes are accepted as sleeping sites under the condition that they are firmly attached to a side of the cage. Such elevated retreats can save rodents from drowning in the event of cage flooding.

It is not the general view at the facility I work, but I personally think that an appropriate shelter should be considered basic cage furniture. The majority of our breeder rats prefer rectangular PVC tubes over round pipes, probably because the pipes are not stable enough for quiet resting or sleeping, but easily roll over when the animals play on them and when the cage is moved. Pipes are accepted under the condition that they are firmly attached to a side of the cage (Figure 31).

I also consider a shelter a *must* for rats and would concur that the animals like small openings that the occupants can "plug" with their rumps. We use cardboard boxes or recycle old polypropylene mouse cages. Both are well accepted by the animals. I have never seen competition or aggression between rats over access to the shelter, although I am always careful to make it big enough for everyone to fit. They usually huddle together in it and very rarely sleep outside, even when they live in relatively large groups.

4.14.3. Hamsters

Our hamsters receive wood-wool, which they quickly turn into fantastic nests. Sometimes animal care staff also provide them with little cotton nestlets, which can be torn up and incorporated into the wood-wool structures. The hamsters are quite content in these nests. I say this because there is no movement in these retreats when personnel enter the room, whereas hamsters without access to such a secluded nesting area get very disturbed and desperately try to hide. I think it is very important to offer hamsters the option of hiding from the human potential predator. Hamsters tend to get hyperaggressive when they are kept in barren cages.


Gernot Kuhnen

Figure 32

Short PVC pipe sections provide appropriate shelters for hamsters.

An appropriate shelter, offering the caged hamster a "safe" retreat, can mitigate this problem (McClure and Thomson, 1992). They seem to really enjoy short PVC pipes (Figure 32). They typically tip them over and then sleep curled up inside.

4.14.4. Guinea Pigs

Like hamsters, guinea pigs have a strong need to hide from the human predator. Their feeling of security depends on access to a covered refuge. PVC pipe sections provide great shelters. Group- and single-housed animals hide in them, run through or jump over them. I am sure they would prefer cardboard boxes, which they could gnaw and which would not roll over, but many of our researchers are concerned that the animals might ingest some of this easy-to-gnaw material, which then could exert an effect in nutritionally sensitive protocols. We have found no evidence that the animals gnaw the PVC pipes.

We use old polypropylene mouse cages with a hole cut out of one wall. They can be removed easily or flipped over when you need to get hold of an animal. Our guinea pigs use these shelters often, especially when people enter the rooms. I do recommend shelters for guinea pigs, because I see the animals making use of them so much, not only for taking refuge and sleeping in them, but also for sitting on top of them to get a better view of the room.

4.14.5. Rabbits

Even though rabbit pens are often furnished with wooden or cardboard boxes, there is no published evidence showing that the animals—with the exception of nursing does—make good use of such boxes as shelters. When we give our rabbits cardboard boxes, they spend a great deal of time sitting or stretched out on top of the shelters rather than resting in them. Once we cut holes in the walls, the animals use these boxes also as shelters and lie inside, looking through the holes.

We house females rabbits in groups of nine and have noticed that, if there are not enough hiding places available, fights are bound to happen. We found that the frequency of such fights is reduced when the animals have access to cardboard boxes that have an entrance and an exit. The doe who chases another doe usually calms down the moment she loses visual contact with her victim disappearing in such a refuge.

4.14.6. Conclusions

The needs of mice and rats for a shelter and nesting material are quite different. Building their own nests is almost a "must" for mice, and the nest will then also be used as a retreat. For rats, access to a solid shelter has high priority, and a nest will be constructed in it when the appropriate material is available; if it is not available, an unfurnished shelter will do. The general well-being of hamsters and guinea pigs is dependent to a great extent on hiding from humans. Rabbits tend to use shelters more as look-outs than dark refuges. In group-housed rabbits, such refuges can help avoid aggressive chases.

4.15. Bedding for Rodents

What is the most appropriate bedding/litter material for rodents?

We use ¾ "dust free" autoclaved softwood sawdust for *all* our rodents and have not encountered health-related problems in any species or strain, including nude mice. We switched to that substrate after quite a number of our nude mice had developed conjunctivitis on fine sawdust bedding that had a relatively high dust content.

Mice prefer shredded paper and wood-wool over woodchips or sawdust probably because the paper and wood-wool not only serves as bedding but can also be used as nesting material (Blom et al., 1996; Eskola and Kaliste-Korhonen, 1999). Carefresh bedding, which is made of recycled paper, absorbs urine and odors well, is nice for bedding and does not cause skin or breathing problems that some of the wood-based litters do. Another advantage of carefresh is that the mice can build elaborate nests with it.

We use corncob litter which also absorbs urine pretty well. There is no indication that it irritates the skin of our mice, who use the corncob litter not only as a bedding, but also as a foraging substrate.

Having tested different types of bedding for **rats**, I think there will be little debate when I say that woodchip bedding is the worst. Corncob and smaller wood flake bedding is not too bad, but I like the compressed paper chip the best because:

- the cage dries out better—better for the animal,
- the cage requires less changing—better for time management,
- there is less dust—better for the animals, workers, and air handling system.

You have to be careful with **hamsters** when you give them paper bedding. Hamsters, who are not familiar with paper, will chew and store it in their cheek pouches where it can get stuck easily. Starting already early in life, our hamsters get paper which they do not try to eat, but consistently use to build a nest. When they are adults, there is no risk that they will pouch paper material.

Paper-based substrate seems to provide the most appropriate bedding for mice and for rats.

4.16. Beds for Dogs

Does anyone supply some kind of bed for singly housed dogs?

I have the feeling that traditional cages are "uncomfortable" for a dog when he/she wants to rest. I would assume that dogs prefer to sleep in a partially closed-in area—against a wall or in a corner—giving them a sense of security. The addition of two, maybe 5 cm high, Plexiglas barriers to the inside of the cage could perhaps create such a secure rest area for a dog.

This is a great idea! I wanted to do something like that for a long time. I will never forget one experience that showed me that dogs want a "bed" to sleep in: We were switching some runs and it happened that we let the dogs stay a few days in the pig room that was vacant at the time. The pigs' empty food bowls were still there. When I walked into the dogs' temporary quarters the next day, I found almost all of them curled up inside these bowls; it was so cute! The dogs showed me very clearly that they appreciate this type of security. After all, they are den animals and appreciate small spaces, just as pet dogs do, who like to go into their crates to hang out and sleep.

An easy way to make a bed for dogs is to buy plastic dog kennels and use each half as a bed. It provides three sides that are high enough to give the animal a sense of security. Our dogs seem to be happy with these "beds."

There are practical options to provide dogs with a "comfortable" place on which they can rest and feel relatively secure.

4.17. Vertical Space Enhancement

Do caged animals benefit from elevated structures?

4.17.1. Rodents

I have designed for my "leftover" research **mice**—aka my work pets!—a multi-level caging system, by stacking a standard long mouse cage into a standard rat cage

Tamara Godbey

Figure 33

This is a species-adequate, custom-made multi-level caging system for mice.

and drilling a single hole in the floor of the top cage, thereby providing an artificial underground space with a deep layer of woodchips (Figure 33). I think it really provides a much improved environment for the mice, allowing them to hide, tunnel and dig, and sleep in seclusion in the lower level during the light hours, and engage in various activities, including wheel running, in the upper level during the dark hours of the day. When they hear me enter the room, the mice always come to the top cage for treats. When they have pups, they keep them strictly in the bottom cage.

The cage arrangement that you describe is ingenious! Your observations strongly suggest that mice—I am sure rats also and maybe even guinea pigs—would benefit from having access to two different levels in their cages, a low-level secluded area for resting during the light period, and a high-level activity area for the dark period of the day.

Nelson et al. (2003) found that **rats** spend only 22 percent of a 24-hour day on raised platforms. The low attraction of platforms is probably because they expose rather than shelter these prey animals.

4.17.2. Dogs

All our dogs have access to an elevated resting surface. We have mounted a little platform on one side of each cage. It can be flipped up against the wall, so that there is more room when we clean the cage. This simple system works well for us. The dogs seem to like their platforms, jump on them and have a good view of what is going on in the room or sleep on them (Figure 34a,b)

Raised resting surfaces are liked by dogs. They provide some degree of security,

Aziz Cetinsu

Aziz Cetinsu

Figure 34a,b
This elevated resting surface for dogs is a custom-made platform that can be flipped up against the wall to create more room during cleaning and to allow the water to run off quickly from it (a). Dogs will "even" sleep on their bench (b).

increase the dog's ability to view outside the cage, and increase the overall area available to the dog. I have noticed that dogs who have access to a platform are more approachable, friendly and playful.

4.17.3. Primates

Primates are biologically adapted to spend most of their time—especially the night—above the ground. The vertical or arboreal dimension is safer for them and, when having the choice, they will spend more time on elevated structures than on the ground both in the wild and in captivity (Bernstein and Draper, 1964; Bennett and Davis,

Richard Lynch

Figure 35

This is a play room where two male cynos spend most of their time on the highest climbing structures.

1989; Reinhardt, 1992b; Ochiai and Matsuzawa, 1999; Buchanan-Smith et al., 2002; Taylor and Owens, 2004; Clarence et al., 2006; Ross and Lukas, 2006). Providing primates with high resting surfaces, therefore, seems crucial for their overall well-being in the research lab setting.

When I release our cyno males into their play room, they will typically spend most of their time on the highest structures available. They may come down to explore a toy briefly but will quickly return to a "safe" high place (Figure 35).

We keep a group of 18 Japanese macaques in a 13 m high tower that has a 115 m^2 floor space and is equipped with various structures installed at different levels (Figure 36). Systematic observations revealed that individuals spend on average more than 80 percent of the day time on structures 4 m or higher above the ground.

Our group-housed cynos became much more compatible after we installed elevated structures in their enclosures. Nakamichi and Asanuma (1998) and Neveu and Deputte (1996) also noticed in Japanese macaques and mangabeys, respectively, that placing high perches in their pens decreased agonistic interaction, probably because the perches allowed the animals to keep social distances as needed.

Do macaques have a preference for fixed perches versus suspended perches?

Most of the primates' natural environment is "fixed." Even a tree is "fixed;" it's only at the end of branches where a monkey in nature would have the sensation of anything like a swinging perch. A fixed perch is a great thing for a monkey. We used to hang numerous swings and movable raised structures into the enclosure of our group-housed cynos, but we could see very clearly that they prefer the stable perches or platforms. Our animals very rarely used ropes or swings. The only ones using those elements were babies and juveniles.

I gave adult rhesus macaques the choice of sitting on a PVC pipe suspended in

Yoshikazu Ueno Yoshi

Figure 36

At this Frame-Kit Tower for Japanese macaques at the PRI, Kyoto University, monkeys spend more than 80 percent of their time on structures at a level of 4 m or higher. Note the two monkeys on top of the tower.

the center of one section of a double cage, and a PVC pipe of the same diameter mounted diagonally at the same height of the swing in the other section of the double cage. The animals used the perch almost eight times as much as the swing (Kopecky and Reinhardt, 1991). The preference for the perch was probably related to the fact that, unlike the swing, it was a fixed structure permitting continuous relaxed postures rather than short-term balancing. Moreover, the perch, unlike the swing, allowed the monkeys to sit right in front of the cage and have visual control over what is going on in the room.

In the small standard cage, a swing cannot really be used for swinging—there is just not enough room for that—but macaques typically use them to produce a lot of noise, by slamming the swing against cage walls. This is perhaps a great acoustical enrichment for the animals but certainly not for the attending staff!

When they have a properly placed resting surface, such as a comfortable perch, do macaques spend the night resting on them?

Our group-housed rhesus macaques have access to perches at about 1.2 m off the ground. On some occasions, I have checked on them during the night and have always found them sleeping on the perches. I have never seen them sleep on the ground. A similar observation has been made by Van Wagenen (1950) who reported that sitting on a board approximately 1 m off the ground was the favorite position of single-caged rhesus macaques, and that the animals slept on the board at night.

Is it necessary to install resting surfaces as high as possible in the primary enclosure?

Yes, definitively! For example, a platform is very desirable for capuchins, but it must be placed as high as possible so that the monkeys can watch for predators from a safe location. High resting surfaces are used by the animals extensively. If they have blankets or similar texture available, they will sleep on their platform with the blankets pulled over their heads!

In the caging systems we use, there is no bottom tier. All cages are 0.6 m off of the floor. Each cage is furnished with a 1 m high perch, so it is pretty much at human eye level—1.6 m height. It seems to me that the animals feel relaxed when they sit on their perch and can meet me at eye level. A low perch has little or no value as a "safe" resting location from our monkeys' point of view.

What is true for capuchins is certainly also true for other monkeys, simply because all of them avoid ground predators, by climbing up trees and spending the night well off the ground in trees or rocky outcroppings (De Vore and Hall, 1965; Hamilton, 1982; Caldecott, 1986; Altmann and Altmann, 1970; Lindburg, 1971; Roonwal and Mohnot, 1977; Di Bitetti et al., 2000). For monkeys height is a major antipredator factor, determining the location of their "dormitories" in the natural habitat, and the presence of some large trees often seems to be the only limitation to their adaptation to a particular environment (Simonds, 1965; Anderson, 2000). Rhesus macaques, for example, sleep in trees sitting on branches, mostly in clusters of two to three monkeys huddled together (Vessey, 1973). A low perch would be of little value to them in the research lab setting. Yet, the placement of resting surfaces at a very low level is legally condoned by the US Animal Welfare Act Regulations (United States Department of Agriculture, 2002) and also by the US Guide for the Care and Use of Laboratory Animals (National Research Council, 1996). Both texts have included the following clause:

> *Low resting surfaces that do <u>not</u> [emphasis added] allow the space under them to be comfortably occupied by the animal should be counted as part of the floor space.*

This legal loophole is probably the reason why built-in perches or ledges are usually installed at a height of only 20 to 30 cm, regardless of the fact that such a low resting surface can block part of the minimum floor space of standard cages that would be required by an animal to turn around freely and make normal postural adjustments (Figure 37). This situation is very unfortunate for the animals and does not have a parallel in any other country.

Viktor Reinhardt

Figure 37

In American primate research facilities, perches are often placed in such a way that they block part of the floor area that the caged monkey—here baboons—would need to freely turn around.

Viktor Reinhardt

Figure 38

A high perch allows this male rhesus monkey to meet the caretaker at "safe" eye level and sit on a dry place while his cage is rinsed with water.

4.17.4. Conclusions

Under normal circumstances—when the cage is not flooded—rodents do not necessarily benefit from a raised platform, unless it also provides cover. Elevated resting surfaces are beneficial for dogs and primates, especially at times when their enclosure is hosed down and the animals can "escape" to a dry place (Figure 38). Given their adaptation to an arboreal life style, a high fixed resting surface should be a basic furniture of every primate cage.

4.18. Environmental Enrichment for Ferrets

There is very little published information on the species-appropriate housing of ferrets. Can anybody share first-hand experience on this issue?

There have been six ferrets at our facility who have since all been adopted out. It struck me that these animals had very short attention spans, so it was important to have a variety of toys and to rotate them frequently. You do not have to spend a lot of money to make ferrets happy. Empty bedding bags were a great hit! They also enjoyed rolling around small cat balls with bells in them, though they destroyed them rapidly and, hence, needed frequent replacements. They also enjoyed playing "tug of war" with a hanging rabbit carrot toy. A large hanging bird bell fascinated them quite a bit. They seemed to be particularly attracted to the ringing, as they would run over to you, if you jingled the bell. One of their favorite toys was a green gummabone. As soon as one of them would pick up the bone, the others would chase him and try to get it.

From what I have heard, ferrets are commonly housed on gridded floors in modified rabbit or cat cages. We housed ours on aspen shavings in a standard pet ferret cage surrounded by a plastic playpen, the kind you can buy at a pet shop as a puppy enclosure. The ferrets used the litter box only occasionally. They would not drink from water bottles, so we gave them ceramic water bowls and discovered that they also like "fishing." We filled a litter pan with approximately 3 cm of water and put floating and sinking items in the pan. One of the ferrets would actually submerge his entire head! For "hammocks," we used surgical drapes and attached them to the rungs of the cage. Typically, the animals slept in a pile either inside a box or in a clean litter pan. We were concerned that they would climb out of the enclosure and get into trouble in the room, but we found that the only time they scaled the wall of their enclosure was when people were in the room playing with them. They loved being held!

4.19. Environmental Enrichment for Guinea Pigs

Is it too messy to provide guinea pigs with hay on a permanent basis?

In my experience, loose hay autoclaved at 220º F for 5 minutes is the best enrichment for guinea pigs. I have used it successfully for a decade with our animals. They nest and hide in the hay, and they eat it. They will trill when you bring them new hay. Their excitement shows you that hay is a species-appropriate enrichment for them.

We keep our group-housed animals in recycled rabbit-cages with perforated floors. Each cage is furnished with a Macrolon Typ IV rodent cage that has sawdust bedding with a generous layer—about 8 cm thick—of hay (Figure 39). The animals "tunnel," hide and sleep in the hay. On top of that, hay is a favored foraging substrate for them. I do not find that hay creates a mess. Guinea pigs like to have a clean sleeping area. They jump out of the Macrolon cage and defecate and urinate in a corner of the rabbit cage, in which they also find water and food pellets. We have worked with this cage design

Richard Weilemann

Figure 39

An old rabbit-cage, furnished with a rodent cage that is provisioned with sawdust topped with hay, provides species-adequate enrichment for guinea pigs.

many years, and I think the guinea pigs are no less satisfied with it than we are.

Our large breeding groups live in floor pens. For enrichment, hay is placed in plastic barrels that have holes in the bottom. Since guinea pigs love to go under anything that covers them, we mount the barrels on approximately 20 cm high iron legs, allowing the animals to run under the barrel. This arrangement also provides foraging enrichment, and the animals skillfully pull strands of fresh hay through the holes in the bottom of the barrel. The only occasional problem we have had with hay was when guinea pigs were tethered and long blades of hay would wrap around the cannula. We now prevent this by simply chopping the hay for cannulated animals into short (about 15 cm) blades.

Our singly caged guinea pigs have PVC tubes or paperboard oat containers—when the guys are too big to fit through the PVC—through which they run and over which they jump. They seem to enjoy this and do it constantly, suggesting that the novelty effect of these short tunnels does not wear off. Other than that there is really not enough space in the cage to add any other enrichment object. We also try to address their social needs by housing them in transparent cages and arranging the cages in such a way that the animals can see each other. This also implies that they can keep vocally in touch with each other, which they certainly do pretty much most of the time.

Guinea pigs do not manipulate their food, but pick it up directly from the ground with their teeth. This suggests that any toy-like enrichment gadgets that may be useful for rats, mice, hamsters or rabbits serve no purpose for guinea pigs, especially those who are kept in single-cages. My pet guinea pig, whom I adopted after he was released from research, does not care to play with any toy-like enrichment gadgets, but loves to chase my hand and then run away from it. I wish I had the time to do this also with the animals in the lab!

Hay and tubes provide suitable enrichment for guinea pigs. To minimize the distress resulting from being alone (Fenske, 1992; Lazaroff et al., 2006), a guinea pig should always be housed in such a way that the isolated animal can keep vocal contact with conspecifics.

4.20. Environmental Enrichment for Rabbits

What are the most effective, yet practicable enrichment options for rabbits?

Branches provide inexpensive enrichment. The rabbits spend quite some time gnawing at the bark, but once all the bark has been removed, the branch is of no more interest to them. **Hay** is more attractive for the animals and more practicable for the personnel. Our rabbits do not get tired of nibbling and eating this natural foraging substrate. Presenting the hay on the top of the cage is a particularly simple but very effective way of providing species-adequate environmental enrichment—strictly speaking "feeding enrichment," because the rabbits are given the opportunity to engage in foraging behavior. Offering the hay in a "hanging manger" is equally useful (Weaver, 2004).

We autoclave the hay at 120ºC for our specific pathogen-free (SPF) rabbits. The hay does change its color and takes on a smell that is difficult to describe, but this does not seem to bother the rabbits who still eat it with gusto.

Toys, especially durable toys, are of little use for rabbits (Harris et al., 2001; Johnson et al., 2003) unless they are replaced constantly. If you have to replace toys all the time to prevent habituation and subsequent boredom, the question arises, if the term "environmental enrichment" is really appropriate for them. Probably not.

I entirely agree. Enrichment should meet the rabbits' behavioral needs. Durable toys do not meet those needs.

There are exceptions: The rabbits in my charge get a lot of entertainment by pushing metal jar lids along the floor and moving the shavings out of the way.

Yes, jar lids, either loose or suspended on a chain, provide great enrichment for caged rabbits. They show keen interest in these gadgets for prolonged periods of time (Bell, 2000). If one rabbit picks up the lid and drops it—or if a person picks one up and drops it—within moments all the rabbits in the room will come and join playing with the lid. They push it around energetically, thereby creating quite a noise. Small bells hung from the ceiling of the cage, are similarly attractive. Our bunnies love these and will nose and push them during long play periods.

Hollow plastic cat toys with bells inside are also great enrichment gadgets. I guess it is the noise of the bells that makes these toys so attractive. Our rabbits play with them over long periods of time. I also have witnessed the domino affect, with one rabbit starting to play and the other(s) promptly joining. When I pick up the toy and toss it, sure enough, one of them will fetch it—just like a dog—and bring it back to me. I will toss the toy again, and this game can go on and on. These cat toys have been

a big hit and the rabbits never seem to lose interest in them. The bell inside gets rusty after a while, and some of the rabbits chew on the toys and finally destroy them, but they are not expensive and we replace them as needed.

We give our rabbits autoclaved **cardboard boxes**, which the animals use not so much as hiding places but as lookout posts. They spend much time sitting on top of the boxes and spy out the land, but they also tear holes in the sides and then spend hours playing tag in and out of the holes. When the box finally collapses after about a week, we just throw it away and replace it with a new one. Our staff saves boxes, so this kind of enrichment costs nothing apart from the effort of collecting and distributing it.

If they can trust you, rabbits enjoy **human contact**. The rabbits in my charge climb on anyone who visits them, pets them and distributes treats. This is a perfect form of entertainment, not only for the rabbits, but also for the staff and students who volunteer to socialize with these animals.

Hay provides the perfect environmental enrichment for rabbits. Objects that the animals can push and that make some noise, while being moved around, can entertain rabbits for long periods of time. Regular positive interactions with humans provide optimal social enrichment for rabbits.

4.21. Environmental Enrichment for Pigs

What kind of environmental enrichment works best for pigs?

After hearing a recommendation of chain-**toys** for pigs, I made my own: I use about 75 cm lengths of heavy metal chain with assorted dog toys attached to the middle or end of it. The toys include Booda rope, the pigs' favorite toy (Figure 40), nylabone rings, kong toys and rubber bones. I attach these toys to the chain with metal clips so that they can be easily removed and rotated from pen to pen. I can hear the pigs rattling their chains and toys when I leave at night and when I come in on the weekends. Whenever I enter the pig runs, I can always see a pig or two with the toys or chains in their mouths (Figure 41). I gave our pigs their toys four months ago. They still use them, and there are no signs that they have lost interest in them. It just makes sense that they need something to mouth as they chew on each other all day long—and chew on me when I enter the pen!

It is my experience that pigs display far more species-typical behaviors and are less restless—no longer bang at the door—when they have access to kong toys, hanging rubber tires and cloth strips than when they are kept in barren enclosures. The kong toys are a great hit. We replace them twice per week so that they can be cleaned—pig saliva tends to be very difficult to wash off once it dries onto/into the rubber material. I have to come with a new kong toy, *plus* scratch the pig, so that she reluctantly releases the kong that I need to get out for cleaning. Rubber tires or cloth strips also provide great enrichment, however, I have noticed that pigs housed on

Tamara Godbey

Figure 40

These are custom-made suspended toys for pigs.

Tamara Godbey

Figure 41

Chewing a toy is probably more species-appropriate than stereotypically chewing pen fittings.

crates, rather than bedding, tend to lose interest in these items. For these pigs we rotate the tires and strips about every ten days to enhance novelty.

Each of our pigs has access to a 15 cm deep wooden tray filled with sawdust that we top every day with fresh **straw**. The animals spend more time "rooting," chewing the straw and playing with the straw than they do with any of the toys we have ever given them (Figure 42). Straw seems to be the perfect enrichment substrate for them, and there is no indication that they will ever get bored from it. Spoolder et al. (1995), Whittaker et al. (1998) and Scott et al. (2006) have shown that the provision of straw prevents the development and reduces the incidence of stereotypical oral activities, such as chewing pen fittings, in pigs.

We have attached a "scrubbing **brush**" on the side of every pen, so that our pigs

Diane Halverson

Figure 42

Straw provides optimal environmental enrichment that pigs do not get bored of over time.

can scratch those itchy spots! Since most pens are smooth stainless, or the walls are smooth tile, the pigs usually do not have an opportunity to rub against anything. The scrubbing brush does not lose its attraction over time, probably because it offers great relief from itching. Our pigs also get discarded linens, which they like to shake and carry around but, fortunately, never try to ingest.

Well-treated pigs don't get tired of **human contact**. Our animal techs spend quite a lot of time just "popping" in to say hello and to give their animals a scratch, which they always seem to appreciate a lot.

Within the given constraints of single-housing, straw provides optimal species-appropriate enrichment of which pigs do not get bored, because it allows them to engage in rooting, foraging, chewing and playing. Toys are best suspended with chains so that they do not get in contact with the manure. Given the strong social disposition of pigs, human companionship is probably the most appreciated form of environmental enrichment for the singly housed animal.

4.22. Environmental Enrichment for Fish and Frogs

Just curious, is anyone providing enrichment for fish or frogs?

Our frogs get PVC tubes in which they hide upside-down plastic boxes, rocks and bricks on which they climb, and plastic litter boxes filled with water serving as little pools. Brown and Nixon (2004) tested frogs in tanks that were empty in one half and furnished in the other half with plastic pipes, an upside-down plastic box with entrance, plastic aquarium foliage, rocks, wood, lid cover or gravel. The frogs showed a clear preference for the tubes, followed by the foliage, the rocks and wood, the box and finally the lid cover (Figure 43). They were not at all attracted by gravel.

Figure 43

Tubes and floating foliage provide species-adequate shelter for Xenopus laevis.

M.J. Brown (reproduced with permission of the Institute of Animal Technology; published in Animal Technology and Welfare 3, 87-95, 2004)

For our fish, we place PVC pipes in the tanks. We have bottom dweller-type fish. They get really spooked if they do not have a place to hide. It also helps with males, who are territorial, but you have to place enough pipes in the tank to avoid competition. We also float pieces of black trash bags on top of the water to create hiding places.

The best enrichment I can think of for fishes are oxygenating plants, e.g., *Anacharis* and *Cabomba*. These plants release oxygen into the water, and the fish like to graze on them. They yank off pieces as if they were horses in a pasture. The plants also give the fish a more complex environment—navigating through the fronds, etc. I just let these plants float; they will send out roots even without being potted.

Empirical evidence suggests that objects under, in or behind which they can retreat or hide provide suitable environmental enrichment for frogs and some fish species commonly found in research labs.

5. Social Housing

5.1. Pair Formation and Pair-Housing of Monkeys

How do you go about pairing previously single-caged monkeys to address the animals' need for companionship?

5.1.1. Adult Cynos (Cynomolgus/Long-Tailed Macaques)

I have had great success with pairing cynos. For some reason, adult males have been much easier to pair than females (Figure 44). Cynos don't always group well, but they make pretty good pairs!

I usually start with a clear, transparent panel between the two intended partners. From this I can usually gage how the socialization will go:

- Attacking the panel = bad.
- Lip smacking or showing curiosity = good.

Richard Lynch

Figure 44

These compatible adult male cynos are engaged in grooming each other.

Most of the time, I know within the first 30 minutes whether things will work out when I eventually give the two individuals full access to each other.

Using a similar familiarization technique, Lynch (1998) and Watson (2002) tested 48 adult male cyno pairs and found that partners were compatible in 94 percent of cases.

5.1.2. Adult Rhesus (Rhesus Macaques/Monkeys)

Is the pair formation technique that we have discussed for adult long-tailed macaques safe for adult, especially male, rhesus macaques?

With slight modifications, I have used this technique successfully with adult male rhesus. I always screened four animals at the same time in a cage arrangement that allowed the animals visual and auditory contact through transparent doors. Dyads who exhibited consistent, unidirectional dominance/subordinance behavior were first allowed simultaneous access to a central activity cage, while still maintaining access to their home cages, for 30-minute sessions daily for one week. Criteria for potential pair compatibility were:

- no serious fighting,
- no persistent attempts to escape, but
- continued undirectional dominance/subordinance behavior,
- increased grooming and
- cessation of aggression.

Partners of such pairs were subsequently re-evaluated when they had simultaneous access to the activity cage for progressively extended, up to 48-hour, sessions in the course of six weeks. Of 15 dyads tested in this manner, 80 percent (12/15) turned out to be compatible during six-week test periods (Figure 45; Roberts and Platt, 2005). We formed also three adult male cyno pairs in this manner. All three pairs were compatible.

If you consider the circumstances under which the animals are forced to live together, day-in-day-out with no private space, their degree of partner compatibility of about 80 percent is truly amazing. How would our relationship with a loved person develop if we had to live under conditions similar to those of pair-housed macaques in research labs?! Human primates who chose to marry each other and live in an environment that allows for some private space, become incompatible in over 50 percent of cases. I guess, we could learn something from monkeys, who are caged permanently in the same boring environment, just by observing them!

I have formed same-sex pairs of carefully pre-familiarized adult female and adult male rhesus and checked their compatibility over a period of one year: At the time of pairing and throughout the follow-up year, female pairs were compatible in 88 percent of 77 cases, male pairs were compatible in 80 percent of 20 cases (Reinhardt, 1994b).

Viktor Reinhardt

Figure 45

Ray and Max, two rhesus males, have lived together as compatible companions for eight years. The two are assigned to a timed breeding program of a caged rhesus colony.

The PI who does research with our pair-housed rhesus insists that cage companions be separated during the night and on weekends, so that they cannot fight and injure each other while nobody is around. I would love to keep the animals together also during the night, but cannot argue with the PI because I really don't know if that would jeopardize the safety of the animals.

In our facility, compatible companions are allowed to remain together also during the night, on weekends and holidays. This applies for both female and male pairs, as well as for all animals who have head cap implants. It has never happened that we found paired animals injured or bruised when entering their room in the early morning. I think there is no special risk when pairs spend the night together without being supervised.

We also keep our male and female rhesus pairs together 24/7 and encounter no problems related to aggression during the night. Articles by Crockett et al. (1994) and Lynch (1998) make it clear for paired male cynos that partners engage in more fighting, when they are re-united every morning, than when they are allowed to remain together also during the night. It is probably more risky to have companions go through a brief re-introduction procedure each morning than stay together also during the night.

At our facility, after pairs have been established, they are housed together uninterruptedly. This includes male and female isosexual pairs, and each species housed here, including rhesus, pigtails, sooty mangabeys, squirrel monkeys, chimps, and cynos. We have not noticed that paired companions fight during the night, on weekends and holidays when nobody is around.

Based on my own experience with a large number of pair-housed rhesus macaques, I would not recommend separation during nights/weekends/holidays as a preventative measure. Generally, when things are quiet with the people, things seem

quiet with the monkeys! And any time you separate, you run the risks of someone forgetting to re-unite, or re-uniting the wrong animals; and on top of that, it's a lot of work for the staff.

5.1.3. Adult Baboons

How do you establish pairs of male baboons? I currently work with 38 animals, ranging in age between 2 to 6 years. I have paired male rhesus and pigtails successfully but have no experience with baboons.

I have introduced male olive baboons of that same age group you mention. At this age, they are relatively easy to work with. First, I observe two potential partners in a familiarization cage in which they can communicate with each other through a clear Plexiglas cage divider. Good signs of possible compatibility are:

- lip smacking,
- reaching out to one another,
- presenting to one another, but
- no overt aggression.

I always allow several days "howdy" time to make sure that the two animals are well familiarized and establish a dominance relationship, which often is not noticeable until they share the same living quarters. Partners, who got along well with each other as neighbors, are subsequently introduced in another unfamiliar cage where they have no reason to engage in territorial conflicts. I establish new pairs always on Mondays, so I have the whole week to check them daily and assure that they remain compatible.

5.1.4. Adult Vervets (Vervet Monkeys)

Does anyone have experience with the same-sex pair-housing of adult vervet monkeys?

It is my experience that it makes no difference to the outcome of pair formation, whether the partners were first familiarized or not. Adult female pairs are compatible in about 60 percent of cases. We have never managed to house adult males in pairs, unless they were reared together right after weaning (8 to 10 months), in which case compatibility is about 90 percent.

5.1.5. Young Monkeys

Is it necessary to also pre-familiarize potential companions when working with young animals who have not reached the age of puberty?

With juvenile cynos, I usually don't take the trouble of pre-familiarizing them, but simply put them together. I have never had a pair that was incompatible.

I also skip the familiarization procedure with rhesus who are three years old or younger. These young animals spontaneously get along with each other, probably because dominance-subordination relationships are not yet firmly established. When they are over three years, they typically show dominance status ambitions—especially young males—which makes it very advisable to allow them to establish their rank relationships during a familiarization period *before* introducing them as a pair. I am always inclined to reduce the risk for the animals to an absolute minimum, even if it means that I have to invest a bit of extra time.

5.1.6. Paired Monkeys Competing over Food

When monkeys are housed in pairs, is competition over food and perhaps even monopolization of food by the dominant partner a problem? If so, how do you deal with it?

I notice this problem in about 10 percent of our pair-housed rhesus monkeys. I tried cooperative feeding for a while. It works if I come in early enough to feed the monkeys myself. Due to time constraints, however, I typically separate the "problem" monkeys with a solid panel until both animals have eaten their portion. Of course, separating monkeys daily for 15 to 60 minutes isn't ideal.

You don't really need to train or separate the partners. When I started pairing rhesus and stump-tailed macaques in double cages, I noticed very quickly that some animals had difficulties getting access to one of the feeders, because the dominant partner tried to monopolize the food. In some pairs, the subordinate animal got so intimidated that he or she no longer made serious attempts to get food while the dominant partner was eating. The installation of dividing panels with a passage hole close to the back wall of the cage (privacy panels) solved this issue (Figure 46), by allowing both partners to obtain food, each from a separate feeder, without seeing each other. I don't remember a single case in which food competition was a problem after this new cage design was implemented throughout the colony of more than 700 pair-housed macaques.

5.1.7. Conclusions

In order to minimize the risk of injurious antagonism upon initial introduction of two strange adult monkeys, it is advisable to allow potential companions to first get to know each other and establish a dominance-subordinance relationship without option of direct physical contact. This pre-familiarization is not necessary for juvenile animals. Potential food competition between paired

cage mates can be circumvented by designing the cage in such a way that the two animals can each access a separate feed station without seeing each other.

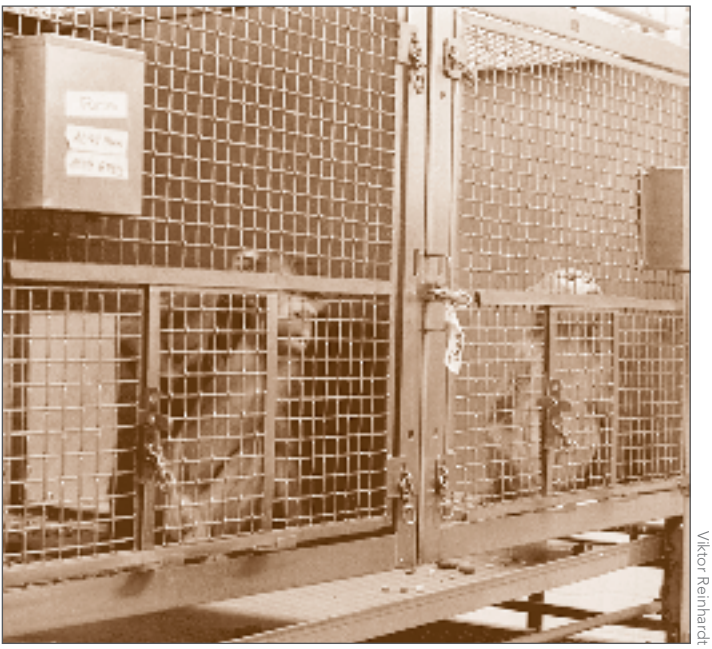
5.2. Sex Difference in Partner Compatibility

Is there a sex difference in terms of compatibility/aggression when you keep animals in same-sex pairs?

It is my experience with rhesus and stump-tailed macaques that male-male pairs are equally compatible—and equally affectionate—as female-female pairs:

- if you make sure that the two sexes have no visual or olfactory contact with each other, and
- if the cage is furnished with a privacy panel so that paired partners can get away from each other as needed (Figure 46).

We keep same-sex pairs of marmosets, and have more problems with fighting between the females than the males. Usually female pairs are okay when they are still young, but when they reach the age of 3 to 4 years, they often start fighting. When this happens, we have to separate the incompatible partners in many cases. It is then very difficult to re-pair them with another female, and we consequently end up with quite a number of single-housed individuals. We have to deal with this age-related social incompatibility also in males, but the incidence is less frequent.



Viktor Reinhardt

Figure 46

A privacy panel with a passage hole at the back wall of the cage allows paired macaque companions to get away from each other's field of vision. This minimizes agonistic interactions and avoids competition over access to the food boxes (Reinhardt and Reinhardt, 1991).

Your observations question the validity of the often-published notion that "males are more aggressive than females." It's true, males may inflict wounds that are more serious when they bite than females, but this doesn't mean that they are more motivated to show aggressive behavior in the social context.

5.3. Making Use of the Stress Buffering Influence of a Companion

There is scientific evidence that the presence of a compatible conspecific can buffer stress reactions not only in people (Bovard, 1959) but also in rats (Davitz and Mason, 1955; Conger et al., 1957; Latané, 1969; Taylor, 1981; Sharp et al., 2002), mice (Goldsmith et al., 1978), guinea pigs (Kaiser et al., 2003; Machatschke et al., 2004), sheep (Fraser, 1995), goats (Pearson and Mellor, 1976; Lyons et al., 1988), and monkeys (Mason, 1960; Coe et al., 1982; Coelho et al., 1991). Do you make use of this stress buffering effect with the animals in your charge?

5.3.1. Post-Operative Care

We have **mice** who are recovering from telemetry-implantation while being housed either alone or in pairs. With several years of experience with this surgery, we now are pretty sure that socially housed mice "feel better" than individually housed mice. Our mice are anesthetized with O_2N_2O and isoflurane. They regain consciousness within a few minutes after surgery, are kept in an incubator for one hour, and then returned to their group mates in a heating mat-provisioned home cage. We have encountered no problems, and it never happened that group members would bully the recovering animal or remove sutures.

I can add here an observation of a colleague who performs spinal cord surgery in **rats**. He lost about 20 percent of the animals when these were individually caged after surgery. Defying tradition, he tried keeping the rats in compatible pairs after surgery. This caused no complications. He then implemented pair-housing for all his post-operative rats. This had the effect that he no longer lost any of his animals. Unfortunately, he has not published this experience and, obviously, does not want to go back to individual-housing to get proper scientific data to support this observation.

It is my experience with rhesus **macaques** that it is advisable to pair-house an animal after surgery as soon as possible with his or her compatible companion. We do this especially with pairs, after one of them had cranial implant surgery. It is the investigator's and my own impression that the animals recover better from the surgery stress when their familiar companion is with them than when they are alone

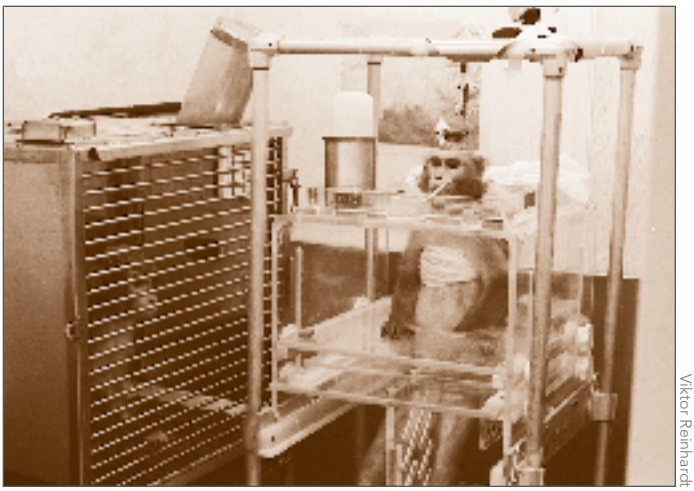
Viktor Reinhardt

Figure 47

Young female rhesus macaque recovering from cranial implant surgery in the company of her adult cage mate who is tethered during an experiment.

(Figure 47). The presence of a companion provides a psychological support that the animals seem to need during post-operative recovery. I should perhaps emphasize the obvious, that we establish new pairs well before surgery and always make sure that the animal who had undergone surgery has regained full consciousness before the companion is brought to the post-surgery recovery cage.

Murray et al. (2002) demonstrated the practicability of post-operative pair-housing in 15 female cynos who were returned to their partners on the day of the operation (placement of vascular access port). Change in hierarchy status, self-traumatic events, weight loss or diarrhea did not occur in any of these animals, and the incision sites healed unremarkably. The animals ate and drank normally, and



Viktor Reinhardt

Figure 48

The distress associated with being chair-restrained all alone in a strange room can be buffered by the presence of the familiar cage companion in a mobile cage.

they accepted their postoperative oral medication without problem.

Close to 95 percent of our cyno population is pair-housed. The animals are subjected to a lot of orthopedic procedures. There have never been problems with the re-pairing of the animals after surgery. We partition the pair's cage with a transparent panel, which we remove after the treated companion has fully recovered from anesthetic effects (usually 24 hours). It has never happened that animals who had no surgery showed any negative behavioral reactions toward their temporarily probably weaker cage mates.

In a small study, we compared post-op recovery of the animals when:

a) only one partner had surgery resulting in a full length cast on one of the legs,
b) both companions had the surgery, and
c) the animal, who had surgery, was kept alone for a few days.

We found that there was:

- less cast picking,
- faster recovery, and
- quicker return to full range of motion after the cast had come off

when the animals were re-paired with their partners, than when they were kept alone after surgery.

5.3.2. Chair-Restraint

When I worked at a primate research facility, my primary motivation for implementing pair-housing was prompted by individual rhesus monkeys, who were assigned to research protocols requiring chair-restraint. These animals were tested alone in sound-proof chambers. Their behavior made it very clear that they experienced anxiety and fear, not so much because they were restrained, but because they were alone—apart from the sporadic presence of the investigator or animal care personnel, who unknowingly frightened rather than comforted the monkey. It took me a whole year to coax the PI into pair-housing all 40+ monkeys assigned to this particular research project. What a difference it made! Whenever an animal was chair-restrained, the compatible companion was now brought along in a mobile cage, allowing both partners to keep uninterrupted visual and acoustical contact with each other (Figure 48). This calmed the restrained monkey, who no longer exhibited behavioral signs of distress, such as open-mouth threat, teeth grinding, restlessness, and refusal of food treats.

If circumstances do not allow conspecific companionship, the attending care personnel with whom the restrained animal has a trust-based relationship can possibly act as a stress-buffering substitute. When my monkeys are chaired during an experiment, I stay most of the time with them, talking to them reassuringly. I have the feeling that my presence has a strong calming effect on them, and this actually is the reason why I do it with consistency with all my monkeys.

5.3.3. Chronic Diarrhea

It is not unusual for a rhesus monkey to develop chronic diarrhea after being removed from his or her social group and transferred to a single-housing condition. I have often noticed that, once an animal has been returned to his or her group, the diarrhea stops. Some cases of diarrhea can clear within a week or two when an animal, who has been kept for a long time in a single-cage, is transferred to a compatible pair-housing arrangement.

We had good success by creating a "chronic group" of ten previously single-caged rhesus who had all been labeled as "chronic diarrhea." They were given pepto tabs in the group for the first couple of weeks, but we slowly decreased as needed. Eight animals were cured by this socio-medical treatment, with no relapse occurring during a follow-up period of over two years.

The fact that transfer to social-housing can sometimes cure previously single-caged macaques from chronic diarrhea, suggests that companionship boosts an animal's immune system thereby increasing an animal's resistance to certain pathogens. There are published reports supporting this hypothesis:

- Alexander et al. (2003) transferred 80 single-caged rhesus macaques to social group arrangements. This change in housing condition reduced the yearly incidence of diarrhea from 20 percent to less than 2 percent.
- Schapiro and Bushong (1994) noticed in a SPF rhesus colony that diarrhea-related problems, typical for single-housed animals, were not as prominent in pair-housed animals.
- Schapiro et al. (2000) found in a subsequent study that the immune responses of singly housed monkeys differed from those housed socially. The authors contended that the affiliative interactions, characteristic for pair-housed monkeys, may diminish the likelihood of severe infection with potentially diarrhea-inducing agents.

5.3.4. Conclusions

Empirical evidence suggests that social animals recover better from surgery when they are not alone, but when a compatible companion is with them. Empirical evidence also indicates that companionship helps rhesus macaques cope with confinement stress, as manifested in chronic diarrhea.

5.4. Capture of Group-Housed Animals

It has been documented repeatedly that group-housed primates can easily be trained to cooperate during the capture procedure (Rose et al., 1975; Smith, 1981;

Taff and Dolhinow, 1989; Reinhardt, 1990; Sainsbury et al., 1990; Luttrell et al., 1994; Kessel-Davenport and Gutierrez, 1994; Klaiber-Schuh and Welker, 1997; Lynch et al., 1998; Mendoza, 1999; White et al., 2000). What about rabbits and rodents? What tricks do you use to catch individuals living in a group, without causing undue disturbance/distress?

5.4.1. Rodents and Rabbits

If you offer rats a food treat, about half the time you pick one of them up for any kind of procedure that is not invasive, they will all come running to you, eager to be picked up and rewarded. This part is simple, but the challenge is to select the right one from the crowd.

I am using the same trick, also with great success. When catching rats in this manner, they show hardly any resistance during subsequent daily injections, a circumstance that drastically decreases injectional wound lesions.

Food reward is the keyword also for mice. They love chicken pellets and will come to the front of the cage to get some, even when this implies that they are picked up, briefly restrained and injected.

Guinea pigs are very skittish when their pen is opened. However, they will predictably run into shelters from which they can easy be retrieved (Gray, 1988).

As for rabbits, they also will come to the front of the cage and allow you to get hold of them, if they can trust you and if they can expect a carrot, a piece of bread or any other food reward.

5.4.2. Cats

Our institution has socially and individually housed cats, all living in large pens. As part of the cleaning procedure, the cats have to move into holding areas and return after their pen has been cleaned. Usually they do not cooperate and have to be caught one by one. Many of them do not like to be handled, so it has been an ongoing challenge to shift them in and out of pens. We've even had a few injuries resulting from handling our more grouchy cats.

This has never been an issue for me. As soon as I look through the window of their room, our cats all perk up and run to the door to meet me. To then catch one of them is nothing very special, and I don't think it upsets any of the cats, including the one that I will have to take out for a procedure. I assume that my success here is based on the fact that I quite often visit the animals, play with them, and do nothing that could make them afraid of me; they trust me.

I recently brought in a laser pointer to play with our cats and soon discovered that

I can prompt individual cats, and even pens full of kittens, to move wherever I want them to move, without catching them but simply by using the laser as a target. I bought our staff laser pointers, and we've found that it's an effective way to move cats for routine procedures. Not only that, but trying to catch the quickly moving "laser prey" is also entertaining for the cats. They get to exercise and play a fun game every day. I've only seen one male cat who is not interested in chasing the laser.

We hang the laser pointer outside the cat room next to the little window in the door, so that passing-by technicians can play with the cats by shining the laser into the room, and enticing the animals to chase the moving light dot. The technicians and the cats enjoy this game, which provides entertainment to both parties. Amazingly, no one walked off with the laser pointer.

5.4.3. Conclusions

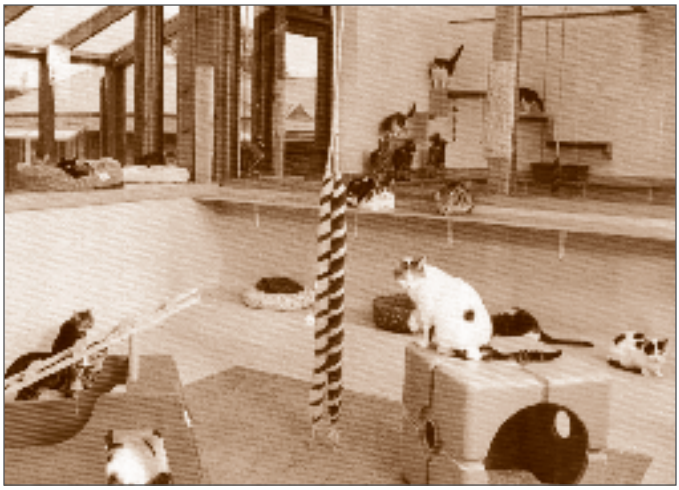
While group-housed monkeys are easily trained to cooperate during the capture procedure, rats, mice and rabbits can be induced to come forward and be picked up by luring them with a food reward. Guinea pigs tend to be more timid but will run into a shelter in which they can then be caught. Cats can be picked up without much ado if they have nothing to fear from you. If they shun you, they will follow a laser point to the location you want them to move.

5.5. Social-Housing of Cats

Cats tend to be rather solitary animals, but seem to prefer companionship—with the option for privacy!—over being caged alone. Is permanent social-housing a species-adequate option for cats in research labs?

We house groups of female cats on a permanent basis; the animals do just fine. Newcomers get integrated without serious fighting. Our cats have access to airline crates, boxes, other hiding places and plenty of elevated resting surfaces (Figure 49). We give them several litter boxes that we exchange daily. In order to circumvent conflicts associated with food, and assure that each cat gets enough, we partition the daily food ratio of a group into more portions than there are cats and distribute them on different locations of the room.

For many years we have kept same-sex groups of up to 18-month-old cats without encountering serious aggression-related issues. Initially, we had more problems with the girls than the boys, but we were always successful in bringing order back into a group of females, by putting a castrated male into their group. We try to keep the groups as stable as possible and, especially,avoid removing cats whom we consider to be the main players in the group. Good care staff, who are encouraged to get to know all the cats in their charge very well and are given extra time to establish good



Geoff Loveridge (reproduced with permission of the Institute of Animal Technology; published in *Animal Technology* 45(2): 69-87, 1994)

Figure 49

Sufficient resting surfaces so that each cat in a room can have her own "private" space avoids competition and possible aggressive interaction.

relationships with them, is a major factor to assure that cats living in groups remain compatible over time.

As long as they are not participating in research studies, our cats are kept in groups in a spacious room. They are all spayed or neutered, a circumstance that makes it unproblematic to keep all of them in a social setting. Bernstein and Strack (1996) kept 14 cats of both sexes (but *all* neutered) in a room that was furnished and managed in cat-appropriate ways, and found that the animals did co-exist "amicably."

Permanent social-housing of cats can be a safe arrangement under the condition that the primary enclosure is properly structured and the personnel committed to providing high-quality care. If all animals of a group are spayed or neutered, the social-housing of cats is relatively unproblematic.

5.6. Social-Housing of Dogs

How are facilities housing their dogs? Specifically, how are you housing pairs and trios and larger groups? Have you found an ideal number of dogs to house together? Are you using bedding material and platforms?

We keep most of our dogs in pairs or trios, but feed them individually to avoid food competition. They all have daily access to a spacious outdoor pen in compatible groups of five to ten dogs. All males are vasectomized. This allows us to house our dogs together regardless of gender, but we do take the precaution of temporarily separating our bitches when they are in heat. In each dog room, we have six or 12 individual pens that can be interconnected as needed. The floors of the pens are solid. We do not use any bedding. Each pen is provisioned with one platform.

The optimal number of dogs per housing unit depends on the breed, and most importantly, the dogs' temperament. Hickey (1993) describes a well-tested, species-adequate caging arrangement and cage furniture for dogs housed in groups of three who are assigned to toxicological studies in which individual food consumption can be monitored.

It seems to be practical and relatively safe to house dogs in small groups of three in convertible runs that allow for the separation of the animals during feeding times and are provisioned with an elevated platform.

5.7. Exercise for Dogs

How do you get a dog to "exercise" in the research lab setting?

It is a legal requirement in the United States that dogs kept in research facilities are given the opportunity to "exercise" (United States Department of Agriculture, 2002). There is, however, no consensus how this can/should be accomplished. To release a dog alone in a large but barren "exercise area" would not be a sensible way of complying with the law. There is no reason to believe that a dog would actually run around alone and play with himself in such an empty, albeit large enclosure (Figure 50).

My current facility uses dogs from Class A vendors. For the most part these dogs don't do much running—unless you run with them—don't pay much attention to other dogs, and rarely play with toys. They mostly enjoy either sitting next to people or being petted. Interestingly, the dogs we have adopted out settled into more typical "dog" behaviors in their new homes: sitting on furniture, running in the yard and

Photo by Hillis Miller, re-designed by Annie Reinhardt

Figure 50
A large but unstructured exercise area is unlikely to prompt a dog to actually exercise, i.e., run around and play.

Novo Nordisk A/S, Denmark

Figure 51

Walking a dog on a leash provides exercise, improves staff morale and helps with re-homing the dogs after research completion.

barking at other dogs. They never, or very rarely, displayed these activities while in the research facility. We've not been able to identify the source(s) of their apparent "discomfort" that causes them not to express more "expected" canine behaviors. They just aren't rambunctious, and getting them to "exercise" isn't an easy task, when they seem much more interested in just sitting in your lap while you talk to them. Putting them on the floor while cage changing/cleaning, or leaving them in a room to play by themselves does not really help.

Campbell et al. (1988) studied beagles in barren enclosures and noticed, not surprisingly, that regardless of the size of the cage, the dogs did not exercise unless people were present in the room. Hughes et al. (1989) concluded from a similar study that human contact is the single most consistent and important factor in encouraging dogs to be active.

Our dogs get daily human attention in a play room. We teach them tricks for treats, groom them, play with them, or just sit with them quietly. Each member of the staff is responsible for one or several dogs, and this includes walking each dog once a day for at least 30 minutes (Figure 51). I can't overemphasize how important human contact is for these animals.

Playing with dogs and walking them on a leash on a *daily* basis is probably the most effective and appropriate option to provide dogs with the opportunity for exercise in accordance with animal welfare regulations.

5.8. Social-Housing of Pigeons

Can anybody on the forum share first-hand experiences regarding the refinement of the traditional housing practices of pigeons?

Our pigeons have been singly caged for as long as 15 years. Not surprisingly, many of them have developed stereotypies such as feather picking, over-preening, head bobbing and circling.

Recently we built a large flight pen and group-housed up to six pigeons at a time. We took the oldest 15 to18-year-old male pigeons first and introduced them in the spacious flight cage. Well, they simply froze; they were terrified! We waited for 30 minutes and then added four females, who had a bit more sang-froid about them and were eager to investigate and hang out with the other birds. It then did not take a long time for all pigeons to settle down and adjust to sharing the big enclosure with each other. They seem to be compatible, and since they live together in the flight pen, I have not noted a single incidence of stereotypical behavior.

Finding the right match may be a challenge, but pigeons—just like any other social animals—do benefit from being housed with other compatible pigeons in a relatively large flight pen, versus being housed alone in small, barren cages.

5.9. The Lone Pig—Addressing His or Her Social Needs

Is anyone in charge of pigs who are kept alone with no other animal in the room? How do you deal with the fact that your pigs are social/herd animals who have a strong need for companionship?

People have successfully used mirrors with sheep (Parrott et al., 1988; McLean and Swanson, 2004) and cattle (Piller et al., 1999), but pigs just don't respond the same way to mirrors. We require regular human interaction for our individually housed pigs, just as we do for our dogs (Figure 52). Someone would go in and sit, pet, brush, even walk the pig.

Weekends and holidays can be very lonely for the pigs in a room by themselves. In the past, we had purchased two mini Yucatan barrows solely for companionship to research-assigned pigs, who would otherwise have been alone. We paid for their per diem out of our Enrichment budget. These two pigs were allowed to move about a large area freely. They became everybody's spoiled pets—it was great for morale! They had a good influence on new pigs assigned to research. The new-comers were always high strung and nervous in the beginning, but after a couple days, seeing us interact with their neighboring buddies, settled in quickly and were soon willing to be

handled by us. We have since retired the two Yucatan pigs and, unfortunately, didn't manage to get replacements yet.

Regular interaction with friendly personnel or permanent visual and auditory contact with another pig living in the same room are good compromise solutions to address the need for companionship of pigs, who have to be single-caged for research reasons.

5.10. Mixing Different Species

Is it a good or a bad idea to keep different species in the same room, or perhaps even in the same enclosure?

It was always my understanding that mice are fearful of rats—who are natural predators for mice—and that stress can be induced in mice by exposing them to the scent of a rat (Calvo-Torrent et al., 1999; D'Arbe et al., 2002). We recently performed a small study in which we assessed urinary corticosterone—as stress indicator—of mice, when rats were present in their room. We did see a stress effect in the mice during the first week. After that, it appears that the mice got used to the presence of rats.

I have housed small rabbits and guinea pigs together, starting out as a pair when they were still very young. They remained together for seven years, often sleeping alongside each other. I have seen problems when large rabbits were housed with guinea pigs. This does not mean that the rabbits are aggressive, but the little guinea pigs are at a certain risk of being knocked over and "flattened" when the big rabbits bounce around in their general enthusiasm.

Scottish Agricultural College

Figure 52

Regular interaction with friendly personnel helps pigs to cope with the distress resulting from being caged alone.

While it may be okay to house different species together it would not be a good idea to keep animals of a prey species together with animals of a predator species—e.g., mice and rats—in the same room.

5.11. Why are Male Mice Housed in Trios?

Why are male mice so often kept in groups of three rather than in pairs?

I've heard that one reason for housing mice in trios is that if one mouse becomes aggressive, he will "share" his aggression amongst the other two males. If the mice were housed as pairs, all this aggression would be released on only one male.

This sounds a bit weird, but who knows? Even if this would reflect reality, would it benefit the quality of research data collected from these animals and, hence, justify the trio-housing? I am wondering if the level of aggression-related stress and the incidence of injurious fighting, is higher or lower in pair- versus trio-housed male mice. If a particular housing system is given priority, there should be hard data demonstrating benefits not only in terms of money—which I assume is the case here—but especially in terms of quality of scientific data and animal welfare.

No scientific data have yet been published that would support the prevailing trio-housing of mice.

6. Stories

6.1. The Bucket Monkey

Many of the messages posted here can be quite serious as we try to find answers to our questions and solutions to frustrating problems. Well, I thought that I would share a funny story to help everyone laugh and take a breather.

We have four rhesus girls, each housed in a large activity cage. *Piglet*—named appropriately!—loves water. She will follow me around as I am cleaning her cage just so she can play with the water jet. She swims too. Yesterday I decided to fill a pumpkin bucket with water for her. Well, for whatever reason she came up with, the bucket ended up on her head! She proceeded to walk around the cage bumping into things and changing direction. She would walk on two legs, then crawl on four. She would do this intentionally. Every once in a while she would take the bucket off, look around and then do it again. She's such a little ham!

6.2. The Rope Mice

I have just had all my beliefs in the sleeping behavior of mice and their preferences for shelters soundly smashed apart when I visited a local pet-shop: Domestic mice were kept in a large cage containing an igloo shelter, nesting material, cardboard tubes, a cardboard box, a wood shavings substrate and a 2 cm-thick hanging rope that was attached to the ceiling of the cage with a hook. Who would like to guess where the two mice were sleeping?

In the open corner huddled together?

On the hook?

Well, the mice were sleeping on the very top of the rope! One appeared to have slung herself over the hook through the knot at the top of the rope, and the other was clinging, but apparently asleep, to the knot at an angle that was almost vertical! These were standard mice being sold as pets—not arboreal miniature lemurs or anything like that! There were plenty of "suitable" sites under cover—which I always thought was the major feature that mice desired for sleeping. I was surprised to see the mice so near

to the lights in the roof, thinking that they would prefer dark areas for sleeping. I would have also thought that, because warmer air rises, sleeping in the top of the cage would not really help them cool.

That's typical for animals: they always prove us, i.e., the human mind, wrong.

Your story just demonstrates so nicely that animals are not little machines but mysterious, unpredictable, fascinating creatures. I vividly remember waking up in our tent several years ago and seeing two little mice curled up right above us in the cup-shaped mosquito net of the tent's roof. They slept in the bright morning light, visible to the birds—and to us—without any protection whatsoever. Why? Because all the burrows were occupied that particular morning?

6.3. The Escapees

I once had a rat escape and get inside an old radiator on the wall. Funny how dumb I was about it. I spent ages trying to reach in, stick things through the ventilation holes to get the critter and cut holes at various points. An hour later it was getting dark, the rat and I were both grimy and annoyed, and we were glaring at each other through the grill of the heater. Finally I stopped and thought: "What do rats like? Places that are familiar, dark and enclosed." I put the rat's home cage near the hole where she had entered the radiator, and turned off the light. Thirty seconds later she was captured and returned back home.

I had a very similar experience with a hamster who not only escaped but disappeared. During the night, the fellow simply gnawed a hole into the wall and dug his way under the floor of the room. You could hear him shoving material out of his way to build a burrow. However, he got hungry, and I counted on that. The next morning, he came up, sniffed the air and headed straight for the carrot, where I could catch him and put him back into his cage, then give him the well-earned carrot.

We are working on a project in which we film mice during the dark phase with infrared light. The technician working on the project is now analyzing the videos from several weeks ago. She told me yesterday that the cameras had caught three mice escaping from a cage—the lid hadn't been replaced properly—then getting back in several hours later! As far as we were concerned, the mice had never gotten out of the cage. We would not have known about this if it hadn't been for the camera. I wonder how many other mice go for midnight walks unnoticed!

I had a chicken called *Roadrunner* who was a terrible escape artist. She could open her cage by finding her way around various pegs and twist ties. Once free, she would lurk around under the cages and slip out when someone opened the door. She would then lurk around in the rafters until someone opened the outside door. I swear she had very definite escape plans; none of this wandering around in plain sight! She got out of the building on several occasions. Fortunately, the building was in a rural area and she only got a short distance before being startled by a sheep and freezing, so I could grab her and bring her back home.

6.4. The Monkey in the Box

We hang boxes—the kind used for organizing small storage items—with a double clip from the tops of the cages of our pair-housed squirrel monkeys. They are a big hit and many of the monkeys spent hours swinging back and forth in the boxes. One pair had a history of one partner "beating up" on the other, stealing treats and pushing him off the perch.

One morning, we heard a terrible screeching, and upon investigation, found the normally subordinate squirrel monkey swinging back and forth in the box with a firm grip on the head fur of the normally dominant monkey. As the box swung back and forth, the poor guy getting his fur pulled was also being pulled back and forth in the cage. We corrected the situation quickly and added a second box, which was instantaneously grabbed by the now dominant monkey, leaving the other box for his partner. This restored peace.

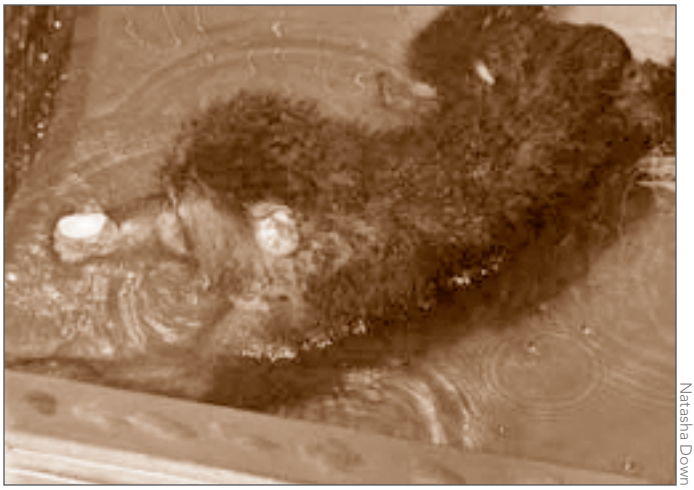
6.5. A Near Accident in the Swimming Pool

We had a near accident in the little swimming pool for our cynos, when an adult female was swimming underwater and a big male started playing around, like a cat chasing after a mouse, from outside the pool and, finally, jumped on the female's back. He put his hands around her neck and appeared to be deliberately holding her under in the 1 meter deep water. After about 15 seconds, I panicked, since I thought he was actually drowning her. I rushed to the scene to "interfere," but just at that point he released her and retreated. She shot out of the water like a rocket and was *really* angry with him, screeching and with rage in her eyes. He looked surprised, and like he had made a significant error, ran screaming away from her as she chased him down and bit him a good one and repeatedly slapped and pinched him. The whole time, he was acting submissively toward her, lip smacking wildly, and ducking as she continued to clobber him.

The two have been in the pool together many times since then, but the female never takes her eye off the male even when she's under water (as cynos dive with their eyes open).

6.6. The Friday Bath

We give our pair-housed rhesus girls a "bathtub" on Fridays. The tub is a rat cage filled with water placed in the tunnel of the two interconnected cages (Figure 53). At first, they didn't know what to do. I then put a carrot in the basin to help them get closer to the water. Soon enough, they were dipping their hands in the water and fished for the carrot. *Kuaui* would sit there and stare at the carrot with her hand above the water.



Natasha Down

Figure 53

Long-tailed macaques love water; they are good swimmers.

Then, suddenly, she'd lunge her hand in and grab it. This girl was full of spunk! In the end, I had them all sitting in the water.

Tejas goes under water and keeps her eyes open, while *Kuaui* dives with her eyes *and* mouth open! Since the rat cage is transparent, I can see everything. The two are quite hilarious! I am surprised they can fit themselves into the bathtub, but they love it!

7. Working with Animals

Animals in laboratories are often scared of people—for good reason!—which makes it difficult to work with them without distressing them at the same time. What do you do to make the animals feel relatively at ease when you work with them?

7.1. Training Monkeys and Dealing with Monkeys—Practical Tips

I am working with several investigators who claim that in order to get macaques to "listen," they first have to "teach" them to be submissive—for example, by intimidating them through shouting. Only then, they claim, would the animals be ready to learn certain tasks during experiments. The idea sounds quite barbaric to me. Is it really ever appropriate to punish an animal?

I have trained many rhesus and stump-tailed macaques to cooperate during various procedures and applied with strict consistency positive reinforcement. You as trainer or handler need to be dominant, not to get the animal to comply, but for your own safety. If the animal doesn't respect you, you are at a risk to be scratched or bitten whenever you interact with the subject. How do you get dominant? Not with a stick, not with shouting, not with impatient reactions, and not with any kind of punishment, but instead with *gentle firmness*. It's a subtle process that I cannot translate into words, but it allows the animal to trust you. That trust is your safeguard against aggression and, I believe, gives the animal more space to comprehend the training tasks.

In my experience, it doesn't help to shout at anyone, including a monkey, when you want to get somebody to do something. Shouting is a punishment, and punishment *blocks* behavior. In contrast to this, positive reinforcement increases the likelihood that the subject will understand what you want and, therefore, show the expected behavior. It is never appropriate to use a punisher to get an animal to do something. It is also never appropriate to punish an animal in order to eliminate a certain behavior, such as urinating at you.

Viktor Reinhardt

Figure 54

An animal made to feel submissive and fearful will not comprehend what you want him or her to do, but rather will try to get away from you.

I very much agree with you: Any kind of intimidation—be it shouting, showing a broomstick or even the net—is bound to have the opposite effect. The animal will feel scared and his or her trust in you will diminish or go down the drain altogether. An intimidated fearful animal, whom you have quasi-forced into submission, will *not* listen to you because he or she no longer feels confident enough in your presence to do what you expect him or her to do. Your negative energy essentially blocks the animal's capacity to learn. It's a losing battle that will make you—and the animal—very frustrated (Figure 54).

What do you do if an animal is very aggressive and you need to protect yourself and attending staff?

We have a male rhesus who often exhibits aggressive behavior to the animal care staff. He tries to grab and scratch them whenever they get close enough. The only way I can place puzzle feeders on his cage or do anything near him is to hold a brush in my hand. The sight of the brush has proven to be "an equalizer." He doesn't try to scratch or grab me as long as that brush is in my hand.

This "equalization" technique sounds fair to me, but the question remains open: Does it "cure" the animal from his misgivings against humans? I very much doubt it. I guess it would help to find out the original reason that made this male so suspicious, presumably non-trusting, and aggressive against humans in general. After all, not all male rhesus are so aggressive. This particular gentleman probably had very bad experience(s) with people that made him so aggressive. I would argue, that:

- animals in captivity *are* not aggressive, but human-created circumstances can *make* them aggressive, and
- any negative reaction to your male's aggression—even the display of

the brush serving as a stern warning—will ultimately reinforce this unacceptable behavior, in accordance with the general rule that "what you resist will persist."

I completely agree that your rhesus male's exhibition of aggression is a human problem and not a monkey problem. How we handle and treat the animals has a lot to do with how they will eventually treat us. The unfortunate thing is that you're dealing with someone else's problem now. We had a similar situation at our facility; this is how we addressed it:

1. We have a 15-kg rhesus male whose mission in life is to scratch anyone or anything that comes near his cage. This one monkey alone used to account for half of all scratch incidents that occurred at our facility. I think he likes the reaction he can trigger in the attending personnel more than anything else, but I must admit it *is* a challenge not to react when a monkey has just ripped your glove and scratched your hand. This can be quite scary when you consider the possible consequences to yourself! It was finally decided that something had to be done about this monkey, and I suggested training him.
2. This monkey loves treats, so it wasn't hard motivating him. Since he could be so dangerous, we had to be very careful working with him. Our cages have small square holes near the bottom. These were the only places we could deliver the treats without being in his reach. We first trained him to sit, which actually came very naturally due to the place we were rewarding him. We gave him a treat only when he was actually sitting down 1) in the front corner of the cage and then 2) would take the treats quietly.
3. The caretaker assigned to do the training worked with this male one or two times every day. By the end of the first month, the monkey was taking treats from the caretaker's hand through the bars without making a fuss.
4. By now, he has stopped his aggressive overtures almost completely. The only time we still have problems with him is, when the room is being washed down and when a strange person is in the room.

Gentle firmness and positive reinforcement are much better training tools than punishment of "undesired" reactions and behaviors.

7.2. Injection and Blood Collection—How to Minimize Stress Reactions

Injection—especially for sedation—and blood collection are very common procedures in biomedical research laboratories. It is my experience with macaques and rabbits that the animals often show avoidance and fear reactions to this

procedure, suggesting that their "normal" physiological status is altered even *before* the actual test or experiment is performed. Are there practical solutions to this problem?

7.2.1. Primates

I have successfully trained two of my singly housed adult **rhesus** males to cooperate during intramuscular injection. As a first step, they learned to present their thighs to the front of the cage and then to be touched with the target, consisting of a small plastic rod. Next, I started gently poking the thigh with the target, then switched to a syringe without needle, followed by a syringe capped with a large blunt needle and then with a normal 25 gauge needle, which I finally inserted into the muscle. I praised the animals at the successful completion of each training session. Both males have learned to cooperate and neither of them reacts in any negative manner to this procedure (Figure 55). I should perhaps emphasize that the two get their injections in their home cages *without* being squeezed. They are in control of the situation, but they do cooperate very well. There is no doubt in my mind that the injection procedure is not a stressful event for them.

I have always found that adult male rhesus react quite well to frequent (once a week) injections if I tell them what I am doing. I show them the needle and I tell them, "I need to give you a small shot." I always talk in a calm soothing voice when I am working with them, and it is not uncommon that they spontaneously present for me, so that I can easily do the procedure (Figure 56). Since the animals show no signs of fear and resistance, injection is unlikely to be a stressful experience for them. After the injection, the animal is praised with "good boy!" or similar phrases such as "you are such a good monkey!" I believe the animals deserve to be approached and handled with respect and trust. They definitely respond better to people they know and trust. Typically, they respond with fear and/or aggression to investigators and to the veterinarian. This implies that I am usually requested to first sedate the animals before the investigator or veterinarian handles them.

With positive reinforcement, I have trained adult female **cynos** to cooperate during intramuscular injection in home cages that are *not* equipped with squeeze-backs. When they can trust you, they readily learn to cooperate during this common procedure. These animals work *with* rather than against me, which automatically implies that they show no fear or stress reactions during the procedure.

I intend to train rhesus and cynos to present for blood collection. So far, I have gained the trust of several animals, but I don't know how far I can trust them in return? I respect them very much—more than I respect some human primates—but the animals are under stress and, therefore, may turn on me for no apparent reason.

It's true, if the animals are under stress while you are working with them, there is a great risk that they will show aggressive reactions to you, in an attempt to get away from the stressful situation. One of the conditions of successful and safe positive reinforcement training is a stress-free work environment, both for the animal and for you. This means, neither the animal nor you should be under the emotional influence of fear, apprehension or frustration. These emotions are dangerous when your handle monkeys or, for that matter, any other animals.

You should reach a stage when you *know* that you can trust the trainee while you work with him or her. This does not mean that you should not be alert, but any traces of mistrust and fear puts you into a seriously dangerous position. Do not work with an animal, unless you have trust in him or her! For your additional safety, you will always have to make sure that your interaction with the trainee will not be disturbed or disrupted by any unexpected event, such as personnel entering the room or loud personnel passing in hallways.

How long does it take to train a macaque to present a leg for a blood draw a) when you make use of the squeeze-back, and b) when the cage has no squeeze-back and the animal is free to come or stay away from you?

Viktor Reinhardt

Figure 55

Rhesus macaques can readily be trained to cooperate during intramuscular injection without being restrained.

Viktor Reinhardt

Figure 56

With some patience, it is easy to train rhesus macaques to allow subcutaneous injection without being restrained.


Viktor Reinhardt

Figure 57
Macaques—here a male rhesus macaque—who have been trained, often cooperate during blood collection in the home cage without the need of a squeeze-back.

My experience might have gone a little differently if the monkeys I worked with had trusted humans, but I had to spend almost an entire month just gaining their trust so that I could touch them. Rather than using a squeeze-back, I used a target to train my animals to come freely to the front of the cage.

To achieve active cooperation in the home cage, I invested on the average 40 minutes with adult male rhesus macaques (Reinhardt, 1991), and 34 minutes with adult female stump-tailed macaques (Reinhardt and Cowley, 1992). These animals lived in squeeze-back equipped cages. They were used to being squeezed for routine procedures, and I also made use of the squeeze-backs during the initial steps of the training. Once trained, the animals showed no behavioral signs of stress or distress prior to and during blood collection, and with many of them, it was not necessary to use the squeeze-back at all (Figure 57). They also failed to show a significant cortisol response to this common procedure (Reinhardt et al., 1991; Reinhardt and Cowley, 1992). I have worked with adult and juvenile rhesus monkeys and noticed that the juveniles—unlike the adults—have difficulties to overcome their fear of being handled. Yes, you can also train them to cooperate during blood collection (Figure 58a,b), but the time investment is considerably higher than with adults (Reinhardt, 1992c).

If you want to employ only positive reinforcement rather than using also the squeeze-back as feels appropriate, you will have to give yourself lots of time to train. Your timetable will not match up to theirs! If training these animals is going to be your main job for the next couple of months, giving yourself four months of training time will probably be sufficient.

I have worked with both single-housed and pair-housed rhesus and got the impression that the pair-housed animals learn faster, perhaps because of the reassurance by the companion.

Figure 58a,b

Young rhesus macaques can be trained to cooperate during blood collection, but it takes them a relatively long time to overcome their initial fear of being touched by a human.

When you have successfully trained monkeys, how do they react to other handlers?

It is my experience with blood collection and topical drug application training that, once trained, the subjects will cooperate also with other personnel, even strangers whom they have never seen, under the condition that the other person knows what he or she is doing and approaches the animal with gentle firmness.

7.2.2. Other Species

I have checked the literature, and I have not found a single publication reporting that any species other than primates have been trained to cooperate during injection or blood collection.

Rodents are the toughest animals for me to give injections without stressing them unduly. There seems to be no way of rewarding them except for their release—so it seems impossible to develop a positive reinforcement training technique for them.

When giving cows injections, I get my best results when speaking softly and taking all the time needed not to rush through the procedure, so that they have a chance to settle down, see where I am and what I am doing. And before I inject, I tell them reassuringly that I am not doing anything that is dangerous for them. It sounds very anthropomorphic, but I do believe that animals pick up on our emotions and intentions and respond accordingly when we are calm versus nervous, kind versus callous, patient versus impatient, and confident versus afraid.

7.2.3. Conclusions

While it is relatively easy to train monkeys to cooperate during injection and blood collection, there is no published evidence that cooperation can also be obtained from rodents, rabbits, dogs and cats.

7.3. Oral Drug Administration—How to Minimize Stress Reactions

Oral drug administration procedures are often stressful and involve considerable risks for the subject, whether he or she is a rat, mouse, monkey, dog or any other species. Does anyone have experience with refinement techniques?

7.3.1. Rabbits

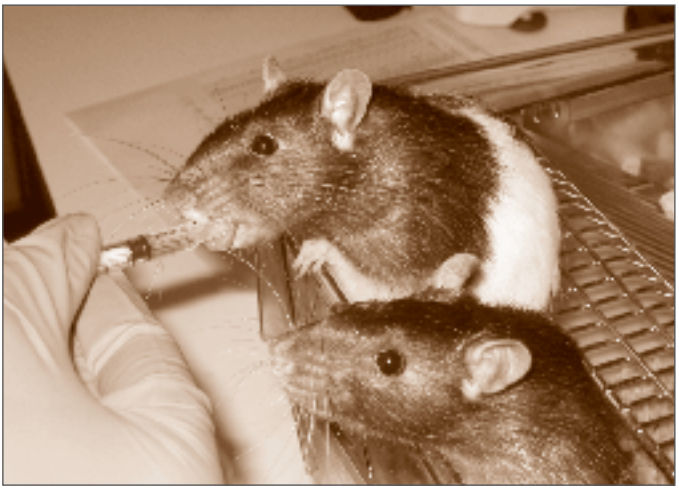
Gavage works well with rabbits. I do not use a gag, but instead hold the rabbit's mouth closed while gently pushing a pediatric feeding tube—with the appropriate length pre-marked—through the diastema. It is easiest to restrain the rabbit in a natural upright position with the neck slightly extended. The animals tolerate this procedure well, even over repeated dosing.

One of our protocols requires that rabbits be given oral aspirin once daily for 30 days. We mix the aspirin with corn oil and flavor this suspension with orange. Believe it or not, the rabbits love it! This kind of oral drug administration is not at all stressful.

Marr et al. (1993) offered rabbits a daily sucrose solution from a tuberculin syringe with a sucrose-granule-coated tip. After five days, the sucrose solution was exchanged with tosufloxacin, but the tip of the syringe remained coated with sucrose granules. Within two days, eight of the ten rabbits willingly took the antibiotic, the remainder requiring minimal encouragement. This procedure was time-efficient, painless and never required more than one technician. It also eliminated physical manipulation, unnecessary stress, and the danger of injury to the animal from improper gastric intubation.

7.3.2. Rats, Mice and Hamsters

I have gavaged rats and hamsters daily for more than six months without noticeably stressing the animals. The success of this dosing method largely depends on your skills and compassion for the animals. I have made it a routine to always offer the animals a



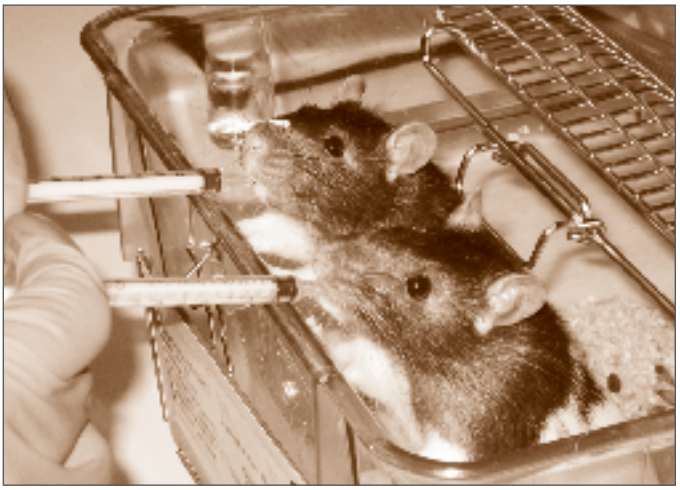


Figure 59a,b

Rats readily learn to drink a 5-10% sucrose-drug solution from a syringe.

Claire Rourke (reproduced with permission of the Institute of Animal Technology; published in Animal Technology and Welfare 6, 15-17, 2007)

little food reward after the gavage. By doing this, the animals will come to the front of the cage, let you restrain them without struggling, hence you can hold them with gentle firmness rather than with a tight grip. Mice seem to be more suspicious. They do not really relax, even when you hold them as carefully as possible, and they usually refuse even the most tasty food reward.

What you describe seems great if not perfect for rats and hamsters. Why do you think mice are more difficult to win over?

I would say that it is a species-specific response to humans. Mice in research will learn to get a treat and come to the front of the cage to receive it, but it is my experience that they never bond with the technician and do not like to be touched. Rats and hamsters give the impression that they like it when you hold them in your hand—even for gavage.

We recently completed a 90-day study of two drugs—indomethacin and celecoxib—mixed in chocolate. Rats like chocolate and this solved a major headache of oral gavage. The rats, living in trios, were first allowed to develop a taste for pure chocolate, by placing a chocolate pellet into their mouth using a 14-gauge gavage needle. After eight days of training, 95 percent of 57 rats displayed eager anticipation of the decoy whenever the cage door was opened. The rats' response did not change when the chocolate pellets contained the test drugs, and they swallowed them without hesitation (Huang-Brown and Guhad, 2002), which means that the oral dosing was not a stressful event for them.

Rats also like sucrose. It took Rourke and Pemberton (2007) only three days to successfully train 12 male rats to voluntarily drink from 1 ml syringes containing a solution of 1 mg donepezil (an approved medicine for treatment of Alzerheimer's Disease) suspended in a 5-10% sucrose solution (Figure 59a,b).

7.3.3. Primates

Our vervet monkeys voluntarily swallow drugs when we mix these with their regular diet, consisting of pre-cooked maize, fortified with vitamins, minerals and other ingredients. The dry ingredients are blended with water and form a stiff putty-like paste, which is an ideal vehicle for mixing in test substances. If the flavor needs to be masked, there are a variety of possibilities, such as honey and syrup, depending on what the protocol permits. We usually administer the compound in about a third of the morning feed. The bulk of the food is offered after this portion has been consumed. Some substances we mix into the entire bulk of the morning feed. Keeping the compound too long in cheek pouches or spitting it out has never been a problem. We have used this simple oral administration technique for pharmacokinetic studies very successfully. Over a time period of 20 years, we have not had to deal with any substance that we could not feed to the vervets, including bitter herbal mixtures in fairly high concentrations.

This is an excellent method! Most facilities have made themselves dependent on commercial dry food, i.e., biscuits or chow that does not leave much leeway for creating a well-flavored paste that effectively masks commonly tested compounds.

When I treated diarrhea with metronidazole—a metallic tasting substance—in a large rhesus colony, nasogastric tubing was the only reliable, albeit stressful, method of administering the drug. Very few animals could be tricked into taking *and* swallowing the drug dissolved in peanut butter, jam, juice, or Ensure. Many of the animals seemed to accept the tablet when it was hidden in a grape, a piece of apple, a piece of banana or in a raisin, but they usually found out quickly what's going on, looked at me, pushed the tablet into the cheek pouch, checked the content of the cheek pouch carefully, and spit the pill out when I turned my back with the good feeling that the treatment was successful. The tablet then made its way to the

sewer—and the animal continued to have chronic diarrhea. I finally habituated the animals to at least "tolerate" metronidazole treatment with the nasogastric tube in a transfer cage. You can even get adult males to sit still while you hold their heads and carefully insert the tube and administer the drug; but you "feel" that the animals are merely tolerating—not accepting—the treatment, and this makes the interaction quite tense and extremely risky for you. I would not recommend it to anybody except in a weekend-emergency case.

7.3.4. Pigs

I have great success in feeding pigs bitter pills such as buthorphanol, diazepam, and antibiotics by using snickers bars and concentrated Jell-O in liquid form—oddly the citrus flavors do not go over as well as strawberry, raspberry and cherry. The key is good acclimatization. If the animals know you are bringing tasty things, they will eat almost anything. Monkeys may be more challenging, as they are perhaps smarter than my piggie wiggies.

7.3.5. Conclusions

With gentle firmness, patience and professional skills most warm-blooded animals—with the exception perhaps of mice—can be habituated to tolerate oral dosing. Rabbits, rats, primates and pigs accept most drugs if these are mixed in specially flavored and specially prepared foodstuff that the animals really like and that masks unpleasant tastes of the drugs to be administered.

7.4. Pole-and-Collar Training of Macaques

I am currently pole-and-collar training one of our adult pair-housed rhesus females and hope that she will graduate to the chair in the next few weeks. *Wendy* does remain sitting when I move the pole towards her but squirms when I try to actually attach the pole to her collar. Can anyone offer some advice how to get over this hurdle?

The adult rhesus monkeys with whom I work also go through an initial period of resistance, when the pole is being attached and also, when they are then put into the chair. But they finally do settle down and cooperate. To start the training, I always first make sure that the trainee is so comfortable with me that she takes treats from my hand. I subsequently include the pole, offering treats with one hand, while holding the pole close to the cage in the other hand. The animals usually get used to this little ceremony very quickly and seem to ignore the pole, while focusing more on the treats.

The poles come with that handy little clip, opening and closing for collar attachment. The clip is a great place to hook treats, which the monkey can retrieve directly from the "dreaded pole." I stuff a marshmallow tightly into the clip. This makes it a little harder for the animal to get the treat and extends the time the animal is in contact with the pole. Once the treat is retrieved consistently without signs of apprehension or fear, I start moving the un-baited pole very carefully in the cage and, finally, also touch the animal with it. In subsequent sessions, I gently tap the collar with the pole, and when I am done hang it on the front door of the cage overnight, so that the animal gets more and more acquainted with it. Needless to say that extra rewards—jackpot if you feel it's deserved!—always are distributed at the end of each training session.

I always collar my rhesus macaques at least two weeks ahead of the first training session, so that they get used to wearing a collar all the time. If they're not comfortable with the collar, it really sets you back, because they will spend most of their time pulling at the collar and scratching their neck. I do not apply any enforced restriction when I train my animals; there is no squeeze-back. The trainee is always in control of the situation. I believe this greatly helps the animals to stay relaxed, keep trusting me and learn quickly what is expected from them in each training session. I consistently reward cooperation with a treat and with praise. If the animal doesn't cooperate, patience on my part replaces the reward. This strategy creates a tension-free ambience.

The first few times the pole is actually attached to the collar can be quite dramatic. The trainees usually freak out the moment they realize what is happening to them. But there is no reason for panic. I simply leave the pole attached and talk reassuringly to the animal who gradually will calm down, stop squirming and remain quiet long enough so that I can carefully unhook and remove the pole. This interaction is always followed by a generous treat reward, which is never refused. During the next sessions, I get the trainee to sit still with the pole attached to the collar for progressively extended periods of time, until she or he forgets all about the pole and takes treats from me. I repeat this step until I get the impression that the animal is comfortable with it.

Coaxing the poled monkey to get out of the cage is always a big challenge. After all, the familiar home cage is a relatively safe haven for these animals. But with patience and many reassuring words, the trainee does finally stop resisting and follows the pull of the pole. After a few sessions, the trainee will feel confident enough to walk—rather than struggle—on the pole and pick up treats from the floor. Should the animal begin to thrash about, I take the pole and carefully but firmly push the animal's head to the floor. To be clear, I do not throw him or her down, but rather use the pole to turn the collar up towards the animal's head and then apply some forward and downward pressure in a determined manner. The monkey is now fixed and can get his or her bearings while being safe from causing any serious problems, such as getting injured while jerking around. I have noticed over and over again that

you can help the animal to calm down when you speak to him or her reassuringly with a gentle whisper-like voice. When the animal has settled down, I carefully start to walk him or her again.

It takes about one week of training until a monkey will cooperate and walk on the pole in a reasonably calm manner and pick up treats from the floor as a reward for good behavior. I want to get the trainees to walk, because after they come out of their cages—or out of the chair—they have a lot of pent-up energy that they like to release, especially the smaller guys. Their legs get cramped sometimes, and they really seem to like the opportunity to stretch. But, I treat this as a reward for good behavior. If they can calmly walk around, I let them do that, but if they start playing "super man," I pull them straight back into their cages. If you don't have enough space, or the racks are enticingly close for climbing and rattling cages, or if you are a little new at this and do not have a second person around who can help you control the monkey if need arises, the pole walking isn't a good idea.

Now, onto the chair:

1. First, push the chair up against a wall with the opening facing out and put all the brakes on. This keeps the chair stable and makes it impossible for the animal to walk straight through, a situation that is really not fun when you're on the other end of the pole!
2. Allow the monkey to explore the chair, touching it, climbing on it, walking around it and perhaps retrieving a treat that you have placed somewhere on the chair.
3. After a day or so, coax the monkey into the sitting position in the chair, and don't forget to reward cooperative behavior!
4. Gently lift the neck into position and get the collar into place. If another person, who is also on very good terms with the trainee, can help you, the situation becomes less of a challenge, especially when you are dealing with one of these incredibly strong and sometimes extremely stubborn guys.

Once you have your monkey in place, let him adjust for a few minutes. Don't forget the treats! Some animals will be initially restless and try to push your hand away, but with gentle patience they will all settle down and finally accept your food reward. Gradually extend the time the trainee remains in the chair, with you always being close by, serving as a comforting social support.

I have found that each "big step" involves an initial struggle, but I have also learned that with consistency and patience, the animals learn quite quickly what I expect them to do. I have a female who is fully trained and just comes up to the front of the cage without being squeezed and actually will move her collar, so the loop is exposed for me to attach the hook of the pole. This monkey also struggled a lot when I first started working with her. It is amazing how these animals can gradually relax into the training sessions and finally start working *with* you, rather than against you. Trust in the trainer is the ultimate key for success. These monkeys are smart and, when they are free of apprehension or fear, they quickly figure out that it is

much easier and even rewarding for them to cooperate with you rather than resist. A successfully trained monkey will have developed so much trust in you that he or she will never fight against you when you pole and chair him or her.

When I train my animals, I work with them daily once or twice, five days a week, until the goal of the training has been achieved. If I don't work with them on a consistent schedule, they tend to get "rusty" quite quickly. The faster you can get them over the initial struggling, the easier the whole training will be. If you try to pole a monkey who vigorously resists on a Monday, and decide to wait and try again on Friday, chances are that the struggle will be the same; but if you are persistent and repeat this training step over and over again every day, you will definitely notice progress by the end of the week. I would imagine that without consistency and patience, the training would be a rather frustrating experience, both for the trainer and for the trainee.

To successfully pole-and-chair train a monkey is not necessarily a time-consuming process. My quickest subject took five days of consistent training to reliably cooperate. He was two years old and an angel! But I also have had some tough customers who have taken me well over a month to get going, especially cranky older females, who can be very stubborn and hard to food-motivate. Also, I have had some animals who were just never meant to be put in a chair. This is a reality that you and the investigators must acknowledge. You cannot force a monkey to cooperate and be relaxed in the chair. It's impossible. Sure, you can try, but you're not going to win.

I think we have to make it very clear to investigators who want us to train their animals that we cannot guarantee to be successful in all cases. Animals are not predictable machines. Yes, most monkeys can be trained but some cannot, or let's say they should not be trained because their personality—which is presumably conditioned through negative experiences with people—is very difficult to deal with. A monkey who persistently resists during positive reinforcement pole-and-collar-chair training is not a suitable candidate for research involving chair restraint. No investigator would benefit from having his or her research subject forced into an experimental situation such as chair restraint. The data collected from such an animal would be of little or no "scientific" value.

I wish all investigators could read this, understand it and accept it!

While strictly using positive reinforcement and applying patient gentle-firmness, most macaques can be trained to cooperate during the pole-attachment-chairing procedure. Some "cannot" be trained, because they have problems overcoming their often-legitimate mistrust of humans.

7.5. Catching Animals Who Have Escaped

What is the best strategy to capture animals who have escaped from their primary enclosure?

7.5.1. Monkeys

I was told by my supervisor that you have to chase escaped **macaques** until they get so exhausted that they will voluntarily go back into their cages. Supposedly, such a stressful experience will make it less likely that they will escape again in the future. I remember a student who was scolded for using an apple—since it was a "reward"—to lure a female rhesus back home after we had chased her around for 20 minutes. The monkey ate the apple and finally walked into her cage. The problem with using so much negative reinforcement was that it typically created quite a chaotic situation. Sometimes the animals in the cages got so excited that they started fighting with the escapee or even with their cage companions. We then ended up, with the veterinarian not only taking care of the injuries of the escapee, but also of fight wounds of other monkeys in the room.

I am staggered to hear that you chase them until they drop. A far better approach is to remain calm and quiet, preferably with only one person in the room. Since monkeys normally retreat from you, it's quite easy to make them move away from you into the direction of their home cage. It is my experience that they are usually only too pleased to get back home.

We had a singly housed rhesus male get loose this morning. He is one of our more grouchy monks with an attitude! He sat on top of the cages and made aggressive overtures towards me and my coworker. On two occasions, he instigated squabbles with some of the other monks in the room, but we were fortunately able to redirect his attention. Finally, through patience, nerve and a lot of praying to the macaque gods, we got the male to jump into an empty top cage into which we had thrown a bunch of fruit. It took about 15 minutes for this to happen. I was so relieved!

I have learned with group- and single-housed rhesus and stump-tailed macaques that catching animals who got loose can be a traumatic and chaotic event, but that it all depends on the personality of the attending care personnel. Some people freak out and create a real mess, shouting, scaring the escapee with broomsticks or trying to catch the escapee with a big net, while other people remain calm and quasi-mesmerize the disoriented monkey into entering a transfer cage, returning directly back into the home cage or jumping into an open empty cage baited with favored food.

Monkeys presumably escape not because they really want to leave their familiar home environment, but because something alarms them, such as an investigator

Viktor Reinhardt

Figure 60

A good relationship with the monkeys—here a male rhesus macaque—and some basic knowledge of their behavior makes it relatively unproblematic for the attending care person to make an escaped animal go back to the home cage.

trying to grab them with heavy leather gloves through the partially opened cage door. If they can trust you and you give them a chance to settle down, they will find their way back "home" without much coaxing—and you close the cage door, while praising the relieved monkey (Figure 60).

We had over 40 rhesus monkeys get out of a corral, because a big branch fell over the wall, creating a perfect ladder. We noticed the situation first thing in the morning, so no one knew how long they were out. The reaction of the caretaker crew was to grab nets and dart guns. My thought was, "Are you crazy? The monkeys will all disperse; they know what nets are for." So, I convinced them to let me fill the corral with fruit and wait some time. And not surprisingly, within only a few hours, every one of the escapees jumped back into the corral and snatched a fruit. No one got distressed or injured. It was all so simple!

When one of our **baboons** escapes, we only have to place fruit in his open home cage. This always works, the escapee returns promptly, and the only thing we have to do is to quickly close the door of the cage. We stand as far from the cage as possible and toss the fruit into the empty cage, and then retreat, so as not interfere with the animals' route back home. We do have nets and a sedative dart gun but, fortunately, never had to use them.

Many years ago, I had some experiences with escaped **squirrel monkeys**. If you tried to catch a monk, the animal would inevitably hop across cage tops onto the floor, back up on top of a cage, across the cage tops, onto the floor, etc., predictably moving in the same pattern. The goal was then to keep the escapee going, with

person #1 trying to catch him or her with the dreaded leather gloves. I—person #2—would don my gloves, memorize the route, stay out of the monkey's path, pick my spot, get my timing right, remain motionless, and only then make my catch. Being preoccupied with leaping and running around and being focused on person #1, an escaped monkey doesn't seem to actually see me. I would make my catch at the base of the tail and gently swing the monk into the waiting hands of person #1. It always worked, and I must confess, it was fun!

7.5.2. Rats and Mice

When rats or mice get out of their cages, we normally use a dustpan if the animals are scurrying on the floor—which they do most of the time. Most rodents, including guinea pigs, hamsters and gerbils, will run along the perimeter of a typical animal room offering no central shelter area. If you place the pan across the run, facing in the direction the critter is coming from, the escapee will run into it and happily sit there while you pick the pan up and safely and gently slide the animal back into the cage. This simple technique minimizes stress for the escaped rodent, eliminates the risk for the handler of being bitten, and it saves the elderly and arthritic amongst us having to get down on our hands and knees to awkwardly try to catch a swiftly moving, agile little animal. If rats or mice have escaped overnight, we usually find them sitting in the food hopper of a neighbor's cage, finishing off the food they haven't managed to transport back to the home cage during the night. Sometimes their home cage gets so filled up with chow from neighbors, that they can't get back into it. This scenario typically implies that the neighbors have bitten the tail and the feet of the scavenging escapee who, therefore, is relieved to be rescued by one of us.

Escaped mice and rats always try to stay as close as possible to the perimeter of the room. Typically, they are focused on moving and seem to be oblivious of my motionless figure hovering above, fingers poised to make the catch. They will come! And will cooperate! You only need to be patient and believe in your perceived outcome! It always horrified me, when the immediate reaction of the staff would be to move the racks, carts, food barrels, etc., while the rodents are scurrying around. Moving stuff only causes the escapees to run in an "unanticipated" direction, or to simply remain motionless to avoid detection, hence rendering any plan useless. Once the direction of the escapee's travel is established, these items make it easier to poise behind or next to, waiting for the inevitable moment when tail and fingers meet. I aim for the base of the tail and I am determined to be successful the first time. If you miss, plan again, anticipate, be patient, be still and be accurate! The trick is to keep the critters from learning a route that allows them to elude capture. Once they learn what's up, they become very savvy in testing your patience.

7.5.3. "Popcorn" Mice

We have a "popcorn" mouse issue. Investigators, using these jumpy little guys, are complaining that they are not breeding well and that mortality of adults is high due to their tendency to jump over staff shoulders as soon as the cage is opened. Husbandry staff are accidentally killing about one mouse per week. Your spontaneous reaction, when these critters start popping out of the cage, is to shut it quickly, which can unfortunately catch these fragile mice between the lid and the cage. I advise everyone dealing with 2 to 3-week-old mice to adopt a mellow, confident, quiet state of mind. The normal reaction is to think, "Oh no, a popcorn cage, here we go again!" and get nervous and impatient. It pays off when you can fight this reaction and stay calm and confident.

Our veterinarian came up with the idea to change "popcorn" mice cages inside a tall Rubbermaid barrel. Now, this isn't easy nor very comfortable, bending over a 1-meter barrel, but it *does* prevent escape! The mice can only jump about two-thirds of the barrel height.

I find paper or plastic tubes for enrichment very handy. It is my experience that a family of ten mice will shove themselves into a tube, making it unproblematic to relocate *all* of them to a new cage by just moving the "filled" tube to the new cage.

7.5.4. Conclusions

By applying basic ethological principles, escaped monkeys, rats and mice can be caught without unduly upsetting the escapee and other animals in the room. It is advisable to place cages of "popcorn" mice first into a container that the animals cannot jump out of and only then open the cage lid. Inevitably, the mice will now pop out, but they can be readily captured in this container.

7.6. How to Make Sheep Move

I have a bit of an urgent question for all of you. We are having problems leading our sheep from their housing quarters down to surgery. Currently we are using the poor system of hooking a leash around their necks and leading—sometimes dragging—them to the area. We worked with three sheep yesterday and have many more to come in the future. Yesterday's experience was very discouraging: The sheep vigorously pulled away from the lead, thereby almost strangulating themselves. Once they are coaxed into the transport cage, the animals are tied onto the side of the cage, which again makes them freak out. They struggle and try to get free and are at risk of hanging themselves in the process. It is horrible

to watch and I cannot imagine what the sheep are going through! There has to be a better way; I was thinking of trying halters to lead them. That way, if they struggle, at least they will not hang themselves. Our next set of sheep are set for tomorrow. I really do not want to see a repeat of what happened yesterday!

You could consider training one individual as a "Judas" sheep—as used in some abattoirs—leading the test sheep into the crate. As long as you have no "Judas," you may want to make sure that you—the potential predator from which sheep would normally run away—do not try to "lead" the sheep but rather guide the sheep *from behind*. If you can provide a meandering path that the sheep can follow, they will be more inclined to walk in the desired direction.

Slightly squeezing the dock makes sheep move forward. The combination of the halter and the squeezing of the dock will probably be your best option, if your sheep must be handled alone. Sheep are downright terrified to be separated from other sheep, so they *can* hang themselves if secured by the neck only. The halter can be made of soft rope, and should fit over the bridge of the nose and behind the ears.

Sheep are best moved by guiding them from *behind* and allowing them to follow another sheep. If a sheep has to be moved alone, the combination of a well-designed halter and the gentle-and-firm squeezing of the animal's dock is probably the most efficient and safe option.

8. Safety Issues

8.1. Aggression Among Males

In some territorial animals—such as mice, rabbits and guinea pigs—males tend to be rather intolerant of each other. This can make it quite problematic to keep them together as isosexual groups in research laboratories. From the males' point of view, is it preferable to be alone? If the answer is yes, how should the cage be structured to provide species-adequate enrichment, so that the single-caged animal is not affected by distress resulting from chronic boredom? If it is preferable to keep these males in a social setting—pairs or groups—what are the options to minimize overt aggressive interactions?

8.1.1. Mice

Before getting hands-on experience in the animal facility, I read plenty about the issue of aggression in male mice and got the impression that it was next to impossible to house them in groups unless they were littermates. Working together with the animal caretakers in our facility, I have discovered that reality is more complex and also more positive than that. The practice in our animal house is that unfamiliar males, who are to be caged together, will be mixed early in the morning. The caretaker will then keep an eye on the animals for two days. If there is severe fighting, the most aggressive—presumably also most dominant—mouse is taken out, and the poor fellow is then housed alone. As an ethologist, I would predict there would soon be a rearrangement in the hierarchical structure, with a new dominant, perhaps even more vicious male emerging, but the reality is, that this intervention does actually help decrease aggression within the group.

Emond et al. (2003) reported that animal care technicians at their center had started, out of concern for injured mice, separating dominant males who threatened, attacked or chased other males. The effect was so positive that two observation periods were set aside daily to identify dominant mice and separate these when indicated. By reducing or eliminating the number of aggressive acts between group members in the

same cage, this "social conflict reduction program" led to a 57 percent reduction of mice being reported for injuries and death.

Male mice of several strains are particularly aggressive after their cage has been cleaned. They do not attack each other as buddies do in situations of conflicting motivations, but they go after each other in earnest. These little guys do not hesitate to inflict serious injuries on each other if they are not separated in time. Other rodents do not behave in this way when their cages are cleaned or changed.

After cage cleaning, individual mice try to establish new territories by depositing their scent marks on objects, such as enrichment items. Dominant males vigorously defend their territorial boundaries. The cramped space of the cage makes such an endeavor almost impossible, because subordinates have to cross these boundaries all the time. The constraints of confinement, therefore, can be a constant cause of territorial conflicts. The incidence of fighting can be reduced in some strains—not in all!—by placing novel toys, novel shelters, fresh cornhusk and paper tissues into the cages (Armstrong et al., 1998; Ambrose and Morton, 2000; Van Loo et al., 2002), allowing subordinate mice to break visual contact with the most dominant mouse in the cage.

There is convincing evidence that:

- scent marks deposited by other males on objects and on the bedding substrate trigger aggressive motivation (Jones and Nowell, 1973; Mugford, 1973; Gray and Hurst, 1995; Ambrose and Morton, 2000), while
- odor cues adhering to nesting material buffer aggressive motivation in male mice (Van Loo et al., 2000).

This makes it possible to minimize aggression among male mice by transferring used nesting material—not soiled bedding material!—at the time of cage cleaning (Van Loo et al., 2003; Van Loo et al., 2004b).

I think it is important to remember that, even though male mice tend to be pretty nasty among each other, they do show a strong preference for companionship even if this implies aggressive interactions. The proximity of another male is preferred to individual housing, irrespective of dominance, kinship or familiarity (Van Loo et al., 2001; Van Loo et al., 2004a). This indicates that "even" male mice *are* social animals who have an inherent need for social contact.

8.1.2. Guinea Pigs

We have kept some of our guinea pigs in groups of five or six for over a half year together. We have now started noticing an increase in aggression, especially bitten ears. Providing two 30 cm long PVC tubes seems to help with the fighting, but it has not solved the problem.

Based on my own experience with guinea pigs, I do not hesitate to say that these highly social animals are remarkably easy-going with each other in stable groups, especially in such small groups as you are dealing with. Space constraints may be

a serious issue in your situation, but I think that a few behavioral observations will give you a clue what the real problem is and how to fix it. There should be plenty of hiding space for *each* guinea pig of your groups. As an alternative to the two PVC pipes—which already probably take up most of the floor area of the cage—you may consider providing your animals with generous amounts of hay, serving both as a source of enrichment and a hiding substrate that *all* members of a group can make use of. If your animals are competing for the tube in order to be sheltered, they should no longer have a reason to compete when the shelter consists of hay.

Cozens (2006) had to euthanize several males due to bite wounds from fighting with cage mates. When the groups received hay on a regular basis, aggression diminished, and the animals stopped injuring each other seriously.

Agass and Ruffle (2005) addressed the problem associated with bullying by partitioning the cage and splitting the original group of four males into pairs. This modification considerably reduced the incidents of biting.

8.1.3. Rabbits

We had no success keeping male rabbits together after they have reached puberty. Our animals live in pens with outside run and places to hide, but this did not hinder them from viciously fighting with each other. Too little floor space may be the main problem; we cannot provide enough space for adequate social distancing. One buck, being chased by another dominant group member, can run away but is bound to quickly turn around, thereby making it impossible to actually escape from the attacker.

In the wild, bucks tend to stay away from each other and hardly ever engage in interactions other than chasing and fighting. Perhaps, attempts to socially house them are misguided. Castration makes them more tolerable (Kalagassy et al., 1999), but it does not eliminate serious aggression (Raje et al., 1997).

8.1.4. Conclusions

Most strains of male mice can and should be housed in small groups, if they are provisioned with proper nesting material—part of which is transferred with them at the time of cage cleaning—and if enrichment items are consistently exchanged with mouse-odor-free items when the cage is cleaned. Male guinea pigs get along with each other reasonably well when all of them have free access to places where they can get away from each other. To permanently live together in the same enclosure with each other is probably not a species-adequate housing arrangement for male rabbits. Their biologically normal intolerance of each other is unlikely to be overcome by castration.

Angel Vilchis

Figure 61a,b

It would not be fair, let alone smart, to pick up a hamster who is fast asleep (a). It is usually safe to pick up a wide awake hamster in cupped hands (b, right).

8.2. How to Deal with Hamsters

How do you work with Syrian hamsters? I have never worked with these little guys before, but I hear they are nasty!

This is an ill-deserved reputation. Fair enough, hamsters are one of the most nocturnal of the common lab animals. You or I, just like a hamster, may be grumpy if someone wakes us up when we are sound asleep (Figure 61a). A hamster who is awake can easily be picked up with one hand or cupped in both hands (Figure 61b).

I have worked with hamsters for three years and have handled them extensively. I have not had one negative experience with catching and handling them, maybe because I love those little cuties! I always wait a couple minutes for them to wake up, before I handle them. This way they don't get startled. I then let them smell me, and finally just scoop them up. They allow me to do this without any protest. I have heard a lot of people claiming that hamsters are vicious, but I believe quite the contrary—let them handle mice to learn what's vicious!

Waking up a hamster before handling is prudent to give the animal no reason to bite in self-defense.

8.3. Handling of Mice

What is the safest and most animal-friendly way of handling mice?

I find the most important thing to remember is, being calm and quiet and move slowly, otherwise you may excite mice, and they will then attempt to bite


Gernot Kuhnen

you. When training people new to mouse handling, I always emphasize: "Don't be a cat! Do not pounce! Be calm and move slowly!" Most people get the mental picture of a cat pouncing on a mouse and realize that this would naturally frighten a mouse quite a bit.

For the safe handling of a mouse. I would make the following recommendation: With a gentle but firm grip on the base of the tail with your thumb and index finger, turn your hand palm down, allowing the mouse to rest on your knuckles. Mice are much more cooperative if they have a firm base to stand on. I have never had a mouse bite me in this position. If you feel your mice are still frightened, you can use small PVC elbows. Just set one end in the cage, put some of their bedding material in the other end to encourage them to investigate and enter. They rarely tire of entering and exiting the elbow as long as there are familiar smells in it. When they are in the tube, you can carry them around.

Unlike rats, mice are better not handled by the body; it's a bit like trying to pick up a wet bar of soap in the bath.

When wishing to carry out injections, the mouse should be able to stand firmly while you pull her tail gently backwards, pick her up by the scruff, tuck her tail under your little finger, proceed to inject and return the mouse promptly to her cage. A little reward afterwards never hurts a mouse either!

The only thing I would add is that you need to have everything prepared *before* you open the cage, so that you can fully focus on the actual handling procedure and get the mouse back into the home environment without any delay. Unlike rats, most mice do not really acclimate to being handled. They want it to be done quickly, so they can get away from you and back where it's safe. I once saw someone scruffing the mouse and then fumbling to open a syringe packet. Fortunately, he was quick to understand why I was chastising him for that and is now always prepared before he even retrieves the cage.

If some basic, simple rules are strictly followed, the handling of mice is not associated with the risk of being bitten by a self-defensive animal.

8.4. Water Leakage

It is not uncommon that malfunctioning watering valves or leaky water bottles result in the accumulation of water in rodent cages, a circumstance that can have serious implications for the animals trapped in such flooded living quarters. In your own experience, what can be done to fix this problem?

I rarely see this problem in rats, but relatively often in mice. It seems to be worse if the animals are nervous or have litters. Since we have moved all our breeding colonies to a separate unit, where the animals are disturbed very little and kept in cages that are provisioned with shelters and nesting material, the incidence of wet cages has become negligible. When we have an occasional problem cage, we reduce the amount of sawdust and use more shredded paper instead. In my experience, water leakage is primarily triggered when the animals build a nest up against a drinker spout but is rarely due to a malfunctioning spout.

We have taken the following measures to keep the number of animals dying as a result of flooding very small:

1. The drinking spouts have small metal gutters pointing downwards, away from the cage. Whenever a spout starts leaking, the water will drip on the floor, rather than into the cage. It is important to make sure that the nipple and gutter are placed correctly.
2. Occasionally, the animals plug the spout with bedding material. When this happens, the cage will flood. To minimize this hazard, the whole watering system is cleaned by the manufacturer once a year. This is a bit costly, but worth the effort and money.
3. One problem we occasionally encounter is that mice will push enrichment items against the nipple of the water bottle, thereby causing it to leak into the cage. We try to prevent this by fixing the enrichment objects to the cage or lid so that the animals cannot move them around.
4. Careful instruction of the animal caretakers can prevent the following hazards:
    a) When water tubes are left on top of the cage, the animals invariably will gnaw on them thereby causing leakage.
    b) If a cage rack contains both small and large cages, a leak in a spout of a small cage, leaking away via the gutter, may leak into a cage below rather than onto the floor.
5. All cages are checked once a day, *including weekends and holidays*, so no cage is left uncontrolled for more than 24 hours.

We have moved away from an automatic watering systems since going to solid floor caging so as to prevent flooding, although we still get leaky bottles. Mice typically build up a mountain of bedding near the water bottle—not sure why—and that readily causes flooding. There is disagreement among caretakers about the amount of bedding to use. Some reason that a very thin layer—hardly enough to cover the bottom of

the cage—and a 250 ml water bottle will prevent the mice from pushing the bedding up into the sipper tubes, and if the cage does flood, "only" 250 ml of water will be contained in the cage. However, there are still incidences of animals, especially pups, dying as a result of water leakage. Other colleagues argue that 1.5 cm of bedding or more will help keep the mice alive, since the bedding will absorb all the water that leaks from one bottle. But pups are likely to die from this also, since they would be cold from sitting on wet bedding.

We hang plastic tubes and other resting surfaces off the top of the cage, functioning as life rafts so to speak (Figure 31). This doesn't do much for the pups, but at least we save the adults. We are hoping this will alleviate much of the drowning risk, since we did not see any better options at this point. In fact, we are currently working on SOPs (standard operating procedures) that will make elevated furniture, such as tubes, a rule for all rodent cages.

I like the idea of tubes suspended on the side of the cages to keep adult mice dry and warm, but getting everyone else on the staff to agree turns out to be very difficult. Some people have a hard time endorsing anything that looks like environmental enrichment, and tubes fall into that category. I have seen videos of mouse and rat mothers carrying their pups to new nest sites. If a cage was to incorporate an elevated dry refuge structure, I wouldn't be surprised if the mothers evacuated the young from damp substrate to this dry and safe site.

Elevated resting surfaces can save animals from drowning and, therefore, should be regarded as basic furniture rather than as enrichment items for mice and rat cages.

8.5. Wire-Bottom Cages

Rodents prefer solid-bottom cages with bedding over standard wire-bottom cages without bedding (Blom et al., 1993; Schlingmann et al., 1994; Manser et al., 1995; National Research Council 1996; Van de Weerd et al., 1996). Apart from this preference, do the animals show behavioral, clinical or physiological signs that they are more distressed in wire-bottom cages than in solid-bottom cages?

We still use some wire-bottom cages for rats assigned to studies that require the exact measurement of food intake and the animal's waste. Any more than a few weeks, and the animals start getting sores on their feet in these cages. The sores are not infected, but I do think that they are painful and contribute to distress. Thankfully, the researchers finally agreed to limit the time that the rats have to spend in those cages to one or two weeks at the most, after which the animals are housed again in solid-bottom cages with appropriate bedding.

Sore hocks caused by wire-bottom cages jeopardize an animal's welfare. We have seen this problem very often in rabbits and in relatively heavy rats kept in wire-

bottom cages. For this reason, we no longer use these cages. Fullerton and Gilliatt (1967), Grover-Johnson and Spencer (1981), Ortman et al. (1981) and Peace and Singer (2001) found in guinea pigs and rats, respectively, that long-term wire-mesh caging is often associated with pressure neuropathies. Kraus et al. (1994) underlines the high incidence of ulcerative pododermatitis (sore hocks) in rabbits kept on wire-bottomed cages. Sauer et al. (2006), however, claims in a study published in the Journal of the American Association for Laboratory Animal Science that:

> *A board-certified veterinary clinical pathologist determined that there were no clinically relevant differences between rats housed in wire-bottom cages and rats housed in solid-bottom cages.*

I am afraid that this "professional" statement can very easily be twisted and used to keep the standard wire-bottom cage in place, at least in the United States where rats are explicitly excluded in federal animal welfare regulations. It is noticeable that even the Office of Laboratory Animal Welfare (2002) discourages the use of wire-bottom for rodents, especially on long-term studies or in larger and older animals, as it may cause foot injury.

Wire-bottoms jeopardize the welfare of caged animals. A 1999 report showed that more than 80 percent of the rodents in surveyed toxicology facilities were housed in wire-bottom cages, presumably because considerable costs would be associated with a change from wire- to solid-bottom caging (Stark 2001).

8.6. Wood in Cages

Have you ever encountered specific problems when you provide your animals with branches or gnawing sticks?

Our **macaques** have access to branches and gnawing sticks. All the wood first goes through a "quarantine" period and remains in a cool dry place indoors for approximately 2 to 3 weeks. We have been giving our animals natural wood for maybe over a year now with no clinical incidents, but we witnessed a behavioral problem associated with the branches: A juvenile rhesus male decided he was a chimp and chased his mates around the pen brandishing the branch as a weapon. After that we secured all branches on swinging cables!

We encountered a similar complication when one of our rhesus male started using small branches as a beating stick for the rest of the group! We switched to PVC pipes and fence boards because of this brat. However, we still give the animals cherry wood gnawing sticks, which we throw away after a few days and replace with new sticks. I am not aware of any clinical issues related to these gnawing sticks.

Over a period of several years, I provided more than 700 rhesus and stump-tailed macaques with gnawing sticks and branches, cut from dead red oak trees, and

encountered no recognizable health hazards (Reinhardt, 1997). The wooden material is cleaned with warm water daily and disinfected once every two weeks during the routine cage sanitation procedures. After one to six months, the branches and sticks are replaced due to wear.

Our **rabbits, guinea pigs and goats** get branches from non-sprayed apple trees—lightly autoclaved for 3 minutes at 120º F. They love them! We set the size limit at pencil thickness, which makes the sticks relatively soft and pliable. Perhaps this is why we encounter no splinter or digestive issues.

The provision of branches and gnawing sticks does not create fomite or clinical problems if common sense sanitary procedures are applied.

8.7. Swimming Pool for Macaques

The center where I work has several cyno breeding colonies housed in large outdoor enclosures. I am interested in using stock tanks to provide swimming opportunities. I have heard that cynos are adept swimmers, but is there a risk of drowning, particularly for infants? Is there danger of one monkey inadvertently drowning another monkey?

The stock tanks we use have a lip half way up the inside of the tank, so if an infant fell into the water it could easily get back out. We used these tanks all of last summer and half of the summer before without ill effects (Rawlins, 2005). The monkeys who do go under water hold their breath for a surprisingly long time. I have watched juvenile cynos swim with no difficulty. I have never come across one who can't swim. It seems to be an inherent skill they don't have to learn.

We give our pair-housed cynos "bathtubs," filled with 30 to 40 cm deep warm water, a few times a week, and have never encountered any problems other than a lot of splashing. Some monkeys take luxurious baths, others climb on a perch and jump into the water, others sit on the side walls and drag their hands in the water, and others wash their fruit in the water. Usually the monkeys make a real mess within the first half hour, and yes they do urinate/defecate in the water. We empty the tubs after about two hours, if the monkeys haven't done it already themselves—which is often the case.

There are a few published articles on the use of swimming pools for rhesus, long-tailed and Japanese macaques. None of these papers mention any safety or hygienic problems (Gilbert and Wrenshall, 1989; Anderson et al., 1992; Anderson et al., 1994; Goodwin, 1999; Rock et al., 2004).

Empirical evidence indicates that captive long-tailed macaques enjoy contact with water, and that access to shallow water does not cause any risk of drowning.

8.8. Pairing Sedated Animals

Allowing two unfamiliar sedated partners to regain consciousness in the same cage is a way to form new pairs for previously single-caged animals. Based on your own experience, would you recommend this pair-formation technique?

It certainly works with pigs and rabbits. We establish new pairs in this manner on a routine basis with great success.

It also works with baboons: Bourgeois and Brent (2005) placed pairs of sedated, four years old male baboons in the same cage and allowed them to wake up together. All seven pairs tested were compatible. Rough-and-tumble wrestling was observed and dominance positions were quickly established, with all dominance disputes followed by bouts of grooming. During two-week follow-up periods no overt aggression was observed.

We don't use sedatives to establish new pairs of macaques, but it sometimes happens that one partner of a pair has been removed and sedated for clinical or experimental reasons. In this situation, we always make certain that the sedated animal first recovers fully before re-uniting the two companions. If the sedative was injected in the afternoon and the subject is still groggy at the end of the work day, we'll leave the two monkeys separated overnight. We don't want to take the risk that the awake partner possibly "takes advantage" and attacks the companion, who might still not be in full control of his or her body movements.

Using sedation as a tool to introduce new cage mates with each other seems to work well with pigs and rabbits and perhaps also with monkeys under the condition that the animals are carefully supervised.

8.9. Pair-Housed Monkeys with Head Cap Implants

Is it safe to house monkeys with head caps as pairs? Do you form the pairs prior to or after head cap implant surgery?

Our university tries to pair all rhesus macaques regardless of cranial implants. Normally the pairs are established before they have undergone surgery for head caps, but we have successfully paired primates after surgery as well. Over a period of ten years, we have had no incidents of damage to the implants. We have more problems, with coils of head caps breaking, in single-housed than in pair-housed rhesus. The head caps of pair-housed animals are cleaner—as they groom each other—than those of individually caged animals (Figure 62).

We have 10 pair-housed male rhesus and long-tailed macaques with head caps. The animals were 3-to-6 years-old at the time of pair formation. They are presently approximately 10-years-old. Some of them had head caps before they were paired,

Viktor Reinhardt

Figure 62

Paired rhesus macaques keep the margins of each other's head cap implants remarkably clean.

others got them afterwards. It didn't seem to matter. In my experience, pair-housing does not create a risk factor when the animals have head cap implants. In all the time I've been working with these monkeys, they've never damaged one another's head caps.

I have worked with more than 100 pair-housed rhesus macaques with cranial implants and encountered no clinical problems related to the fact that these animals shared a cage with another companion. I always established the pairs prior to surgery, but this was perhaps not necessary. I just didn't want to take any avoidable risk.

Practical evidence indicates that macaques can—and should—be pair-housed, without undue risk of jeopardizing ongoing research, though one or both partners of the pair has a cranial implant.

8.10. Re-Pairing Macaques after Separation

We have several same-sex pairs of adult cynos and rhesus who will be assigned to a project requiring repeated 48-hour separations, during which one partner will be tested in another room. The question is: Will it be safe to re-unite the animals after the testing, and will the pairs remain compatible when they are repeatedly separated and re-united?

Your animals will be separated only for relatively short periods, so I really don't think you have anything to worry about re-pairing them. I had no trouble re-pairing several adult male cyno pairs who were separated for weeks. The only animals I had consistent difficulties re-pairing were adult rhesus macaques of both sexes. When you simply put them together, the two compatible companions may not recognize each other quickly enough at the moment of re-pairing, but treat each other as strangers and start fighting. The consequences of this misunderstanding usually is very traumatic. I finally discovered that you can avoid this risk by

inserting a transparent or grated mesh cage divider, and then introduce the one who had been away into the empty half of the home cage. Let the two find out who they are, and then simply remove the divider. I have used this trick many times without failure.

Re-union of temporarily separated cage companions bears some risk if both partners do not recognize each other instantaneously and, therefore, treat each other as strangers. This risk can be minimized by giving the two partners the chance to recognize each other first through a transparent barrier, and only then re-introducing them.

8.11. When a Monkey is Lying Down

I have heard that monkeys are will lie down when something is wrong with them. I do notice that some of our rhesus macaques spend a lot of time lying after they have experienced a distressing situation, for example after surgery and after enforced medication. I am wondering, should I really be concerned when a monkey is lying down?

I have observed some of our rhesus, bonnet and long-tailed macaques lying down. This happens rarely, but I can say that none of these monkeys was sick. I am like you, however, whenever I see a monkey in a recumbent position, my heart always skips a beat. It's true, the sight of a lying monkey is a bit alarming. I don't really know why.

Some of our rhesus girls occasionally lie down during the day, but there is nothing wrong with them. They either lie on their stomach or their sides. I never really thought anything ill of it. We have one little girl, who likes to lie down in her cage most of the

Viktor Reinhardt

Figure 63

Rhesus macaques often like to take a nap on the highest resting surface of their enclosure, which is the safest place in the event of an approaching ground predator.

time. She doesn't rest in her hammock or on the perch but always on the floor. She is healthy and by no means distressed. She lies down just like you or I would do when taking a nap. If I happen to approach her cage, she'll get up immediately.

We have a rhesus who does the same thing. The first time I saw her lying on the bare cage floor, I was scared to death. I thought she was in serious trouble. It is always comforting that she gets up the moment I walk into the room.

If your monkey had access to a tree, you would probably see her lying on her belly or on her side on a branch. It looks funny, but it's normal. I have seen this quite a number of times in group-housed rhesus who would often climb up on the highest perch and take a nap (Figure 63).

When you have a monkey who remains in a lying position even when you approach the cage and get ready to open the door, this is an alarm signal that you better do not overlook. Otherwise, lying down is a sign of comfort rather than discomfort.

8.12. Retro-Orbital Blood Collection

How safe is the retro-orbital bleeding technique?

I used to take blood samples from the retro-orbital sinus in mice and got quite good at it. Fortunately no multiple bleeds were required on the same day. Now, I am in a different department and need to take eight samples in 24 hours. It really bothers me to use this site at the eye so often. I believe the saphenous vein is the way to go, although it may take longer in the beginning to become really proficient. A person working in ophthalmology told me that he did not like the retro-orbital bleeding method at all because it can easily alter the intraorbital pressure, causing severe discomfort to the subject. So yes, there are legitimate ethical concerns.

To my knowledge retro-orbital bleeding is mainly used in mice, rats, hamsters and guinea pigs. This technique does have important advantages. The technique is:

- quick,
- easy in skilled hands, and
- yields a relatively large sample.

Additionally, the eyes can alternated with a one-week interval, and the rodent subject recovers quickly as reflected in corticosterone, catecholamine and behavioral responses (Van Herck et al., 1994). These practical advantages, however, are outweighed by serious ethical disadvantages:

1. The procedure is painful and, therefore, should never be done without proper anaesthesia.
2. There is a risk of complications, especially forward protrusion of the eyeball, caused by continuous bleeding from the retro-orbital venous plexus. This leads to a gradual drying out and a constant itching of the cornea, as eyelids are no longer able to close properly. The animal will react with excessive

scratching, and by doing so will ruin the cornea. Within a short while you will find the animal with a blind eye.

3. The procedure is esthetically unpleasant.

In Denmark it is forbidden to take blood from the retro-orbital sinus without proper anaesthesia, as the procedure is deemed to be really painful. One has to remember that the conjunctiva has to be penetrated during this procedure. Taking blood from the lateral saphenous vein or by a small cut in the ventral tail vessels can be done without anaesthesia and goes fast in mice and rats.

The first time I saw a retro-orbital bleeding was about five years ago. We needed a sample to test for MHV (mouse hepatitis virus). I called our vet and asked if he could teach us newbies how to get a blood sample from a mouse. He discussed various methods and then told us that he always does retro-orbital bleeding on mice. He then proceeded to do the deed, without any anaesthesia. It took maybe 4 to 5 seconds! I do not mind saying that I went completely weak in the knees and if I had not been standing next to a wall, I might even have gone down! We checked the mouse several times that day and he seemed fine, better than me in fact.

It's true, retro-orbital blood collection appears to be somewhat gruesome, but if you have a good teacher and enough practice—this above all is the most important part of the puzzle—it isn't a bad method. It is quick, provides a good amount of clean sample and, in my opinion, requires little to no anesthetic, depending on how much your mice resist. Now, I will admit that errors can occur during these bleeds, and I myself have made a few that have ended up in a way that definitely did not sit well with me at all.

Clinical and ethical concerns outweigh the practical advantages of the retro-orbital bleeding technique in rodents. Preference has to be given to alternative techniques, especially to the saphenous blood collection procedure, that are less risky.

8.13. Barking Dogs

Barking dogs can be a serious noise problem in research labs. Do you—and the dogs—simply put up with it or do you try to modify the environment so that the dogs have less reasons to bark?

We house 40 to 60 dogs at a time in two rooms adjacent to each other. Whenever we enter a room, the dogs greet us with barking—of course—but they usually chill out and stop barking after a short while, except at feeding time! We require that everybody wears ear protection when working in dog rooms. I wonder if having music in the rooms would help. Kilcullen-Steiner and Mitchell (2001) found that a "white noise" stereo system along with "new age music" can effectively decrease the amount and intensity of the barking.

The dogs at our facility bark much less if they are taken out and walked. We have a volunteer walking program. We also noticed a significant decrease in barking after we placed platforms in all our indoor group-runs. The platforms serve the dogs as look-out sites from which they can monitor all activities in the rooms, especially people entering the room.

It is probably impossible to make dogs stop barking altogether, but there is no need to accept barking that creates a noise problem. Dog-adequate enrichment, especially platforms giving the animals visual control of their immediate environment and regular walks with an accompanying person, can effectively decrease the dogs' need to bark.

9. Extraneous Variables

Extraneous factors that influence research data increase the number of animals that are needed to achieve statistically significant findings (Home Office, 1989; Institute for Laboratory Animal Research, 1992; Brockway et al., 1993). This makes it an ethical imperative to examine and then control these factors as best as possible (National Research Council,1996; Öbrink and Rehbinder, 1999).

9.1. Exposure to Distressed Conspecifics

When you subject an animal to a distressing procedure, are the other animals in the room disturbed?

We have just finished a study on the effects on cage mates, when mice are subjected to one-hour restraint stress in the animal room and then returned to the cage. We did this once daily for 14 days. The cage mates were not touched and had implanted telemetry transmitters to monitor heart rate and temperature. While mice were being restrained, the heart rates of the untouched cage mates peaked at about 650/min, 15 minutes into the restraint period. Only by the end of the one hour restraint period, had heart rates of the untouched cage mates returned to baseline. The untouched mice's stress response did not show signs of adaptation within the 14-day study period. A similar pattern of stress response to witnessing restraint stress of the cage mate was found for the rise in body temperature.

Iimori et al. (1982), Fuchs et al. (1987), Pitman et al. (1988), De Laat et al. (1989) and Guhad et al. (2003) documented that rats show physiological stress responses when they are exposed to a conspecific who exhibits signs of distress during a handling procedure. Flow and Jaques (1997) took blood samples of long-tailed macaques while restraining them in their home cages with the squeeze-back. Serum cortisol and thyroid hormone concentrations differed between control animals and animals who, while waiting for their turn, witnessed how others were physically restrained and sedated for blood collection. The authors concluded that the difference might have been due to anxiety resulting from seeing restraint and sedation of other animals.

At our facility, only non-invasive procedures, such as weighing, can be performed in the animal rooms. Everything else has to be done in procedure rooms. This creates

fewer disturbances for both animals and humans. I realize, there is some stress involved in temporarily moving individual animals away from their familiar quarters to procedure rooms, but I have the impression that this policy reduces the overall disturbance and stress that *all* animals of that particular room experience.

I agree, yet it has been shown in rats (Friedman and Ader, 1967; Brown and Martin, 1974; Euker et al., 1975; Dobrakovavá and Jurcovicova, 1984; Damon et al., 1986; Duke et al., 2001; Sharp et al., 2003), mice (Drozdowicz et al., 1990; Tuli et al., 1995; Tabata et al., 1998), guinea pigs (Fenske, 1990) and primates (Mitchell and Gomber, 1976; Line et al., 1987; Phoenix and Chambers, 1984; Coe and Scheffler, 1989; Crockett et al., 1993) that being removed from the familiar quarters is already a significant stressor, which changes the subjects' physiological equilibrium, thereby invalidating research data. So, whether we

- handle a research subject in the animal room—thereby disturbing many animals, or
- move the research subject to a procedure room—thereby causing additional stress prior to the actual handling,

stress seems to be an unavoidable variable, unless perhaps, we can train the research subject to voluntarily cooperate during the handling procedure in the familiar homecage.

I found in rhesus macaques that animals who cooperate during blood collection in the homecage show neither a cortisol response nor behavioral signs of stress that could possibly disturb the other animals in the room (Reinhardt et al., 1991; Reinhardt and Cowley, 1992).

Being exposed to a distressed conspecific changes the physiological equilibrium of an animal, without the animal necessarily showing this in his or her behavior.

9.2. Construction Noise

Our city is going to build a tunnel running underneath our primate facility. I am concerned that our animals will be affected during the digging, drilling and dynamiting. It is planned to move the rodents out of the building but keep the primates during the construction. How will our monkeys cope with the noise?

We had a similar experience and noticed that our macaques were very disturbed in the beginning, but seemed to get used to the occasional bursts of extreme noise very quickly. It helps the animals to remain calm during periods of extreme noise, when the attending caretaker stays in the room, talks to them and offers favored food or other items that they find attractive.

I work in a monkey facility where the floors in the hallway right outside the primate rooms were recently jack-hammered and a tunnel built underneath the building. The flooring project took four months and the tunnel is still not completed. In my opinion,

the noise is not the biggest issue with our monkeys, though I did notice an increase in locomotor stereotypies during the jack-hammering. What really distressed the animals was the fact that we had to keep moving them around the building, so that the workers could access the hallways to take the flooring up. That did upset the animals quite a bit, and it took them several days to settle down in their new home cages.

Since the issue of noise has been brought up in reference to primates, it made me curious to know, if there is any published information on the effect of drilling and jack-hammering on rats and mice. We will be having drilling and jack-hammering in our facility for about three days in a few weeks. It was decided to stop data collection during that time and move the animals housed closest to that area to another room down the hall. I am wondering if that is enough to protect them from excessive stress?

When construction was being done on campus within an acre of our facility, the barely audible noise and vibration threw all our rodent breeding programs out the window for quite some time.

We had been in the middle of construction off and on for the past 10 years. Most of the construction has had few consequences. However, when they built the classroom building across the street from us, they had to drive pilings and tamp the ground because the building has no basement. For six weeks, the ground vibrated constantly for eight hours a day. We lost at least six months of breeding of the transgenic mice, and even the zebrafish stopped laying eggs.

Mice, rats and guinea pigs show a distinct withdrawal response to experimentally generated intense noise (Anthony et al., 1959), suggesting that the animals are stressed.

I checked the literature and found not a single article, assessing the impact of construction noise on the physiology of rodents, rabbits and primates in research labs.

Given the fact that noisy construction and remodeling work is a common event in biomedical research facilities, it is surprising that not a single article could be found in the scientific literature, assessing the impact of this uncontrolled variable on animals assigned to research.

9.3. Researcher

It is my experience that many principal investigators show little or no interest in how their animals are housed and handled, and if they do handle their animals themselves, they often lack proper skills and patience. How do you "train" such individuals to realize that their attitude defeats sound scientific methodology?

It is quite difficult to "train" researchers to do their work with the animals in a

more considerate and compassionate manner. Unfortunately, investigators often see "the results" of the research as more important than the animals themselves. They are in a hurry to get results. Usually they do not take the time to get to know their animals, let alone work with them in a more relaxed, less stressful ambiance.

One thing that really bugs me about this business is that a lot of the time investigators do not know how to treat the animals as sentient beings. A big problem is that first-hand experience with animals is often not a requirement for the researchers and their technicians to be hired, and/or to receive funding for their research proposals. I wish everyone involved in animal research was an "animal person," but sadly, that is not the case.

I am working with investigators who do not know that the rats they are doing research with are nocturnal animals. I always love the statement "they seem happy to me." I actually hear that quite frequently from researchers. Usually they simply mean that the animal is moving about in the cage, but there is no comprehension about whether the movement is normal or indicative of stress or boredom or discomfort. Reese (1991) aptly observed in the book *Animals in Biomedical Research*:

> *That many scientists lack detailed information about their animals, especially their behavior, is distressing and reflects a serious disregard for the single most important element of their research. The animal is the key to the entire experiment.*

It is so obvious for those who genuinely care for the animals and are concerned about valid scientific methodology, but it seems to be of little or no relevance for those who see the animal merely as a means to get publishable data.

Our investigators usually have grad students doing the research-related procedures. It is not very often that they will show up in the animal areas, while others I have yet to ever see. We had some grad students come in to work with mice, others to work with monkeys, but they had never actually worked with a mouse or a monkey before! Apparently, the principal investigators had failed to make sure that their students had received basic training and were actually qualified to work with the animals in an appropriate manner.

Time is a major factor when dealing with researchers and their attitude towards animals. Often, I have tried to help the researchers with a task involving animals, such as acclimatizing an animal to a restraint procedure, only to be told, "No, that would take too long." There are many things that we could do to help alleviate stress, and improve the well-being of the animals, but these refined techniques may take a bit longer than the traditional, often quite brutal methods. The researchers usually give the impression of being in a hurry to get their data as quickly as possible and, therefore, that there is no "extra" time for the animals themselves. It is my experience that it is exceedingly rare to find researchers who "get into the muck" and have some appreciation of what it requires to provide decent housing and handling conditions for their animals. We still have quite a few who do not even want to walk through our dirty cage area side to drop off empty caging.

It always strikes me that many investigators hardly ever show up in our animal area. Some of them probably have never seen the animals assigned to their projects. Yes, they are familiar with the IDs and the subjects' history, but that is often the end of the "touch." A prestigious biomedical scientist puts it in a nutshell when he concedes that:

> *Most investigators think only briefly about the care and handling of their animals and clearly have not made it an important consideration in their work* (Traystman, 1987).

Researchers, who pretend to be too busy to show an active interest in the welfare of the animals assigned to their studies, cannot assure that the data they are collecting will not be influenced by uncontrolled variables related to species-inadequate housing and species-inadequate handling prior, during and after procedures.

9.4. Workdays Versus Weekends and Holidays

Are the animals in your charge less stressed on weekends and holidays than on workdays?

Our rhesus and stump-tailed macaques, but also our guinea pigs, rats and chickens are less restless, less alert and apprehensive on weekends and holidays than during workdays, when personnel can enter their room any time, catch them and subject them to a painful procedure.

I agree that the animals—I refer to rhesus—seem to be calmer, more relaxed on weekends and holidays. This is perhaps not surprising, because the personnel who do the more invasive parts of the research are here during the week, not on weekends and holidays. Hassler et al. (1989) and Schnell and Wood (1993) assessed cardiovascular stress parameters of rhesus macaques and marmosets and found that values are significantly lower on the weekend than during workdays. Entry of technical staff into the colony could be clearly identified in the heart rate and activity recordings of the animals. Schreuder et al. (2007) made similar findings in rats: The animals' heart rate, locomotor activity and blood pressure differed significantly on workdays versus weekends.

The stress level of animals is higher on workdays as compared to days when no personnel are around. This phenomenon has implications for the interpretation of stress-sensitive data, as these may not reflect normal resting values on ordinary workdays.

9.5. Multi-Tier Caging

The cages of small and medium-size animals—such as rodents, rabbits, cats, monkeys, birds—are traditionally stacked on top of each other to allow maximal usage of room space. Animals caged in lower rows live closer to the ground and in a less illuminated environment than animals caged in upper rows. These differences introduce extraneous variables that are usually not accounted for in scientific articles (Davis et al., 1973; Gamble et al., 1979; Reinhardt and Reinhardt, 2000). Is this an issue we have to be concerned about?

Hens on the bottom tier are often more reactive—frightened?—than hens from upper tiers. Presumably, the birds on the upper tiers have been exposed to the sight of human eyes more frequently and so are more habituated to human presence.

This probably also applies to pigeons. Those caged in the bottom row are definitely more fearful and emotional, especially when I bend down face to face with them. They are harder to extricate from the cage and, certainly, flap their wings more when I interact with them in any manner.

Ader et al. (1991) noticed the opposite effect in mice: Animals caged on the top of a rack are more fearful and more "emotional" than those caged on the middle or bottom shelf of the rack. Mice probably feel more secure and secluded in the relatively dark environment of lower shelves than on the top shelf that may expose them directly to bright light. Garner et al. (2004) found that barbering was significantly more severe in upper-row than in lower-row caged mice. Lagakos and Mosteller (1981) also studied mice and found that the incidence of certain tumors increases conspicuously from the bottom to the top shelf. Similar observations were made by Mantel (1980), Greenman et al. (1984) and Young (1987). These studies make it quite clear that shelf level *is* an important variable that needs to be taken into consideration in scientific research with mice.

It is my experience that macaques living in bottom-row cages show more behavioral stress responses—such as crouching in a back corner, alarm vocalization, hyperaggression—when an investigator, dressed up in protective garb and a surgical mask, enters the room than those living in upper-row cages. At the same time, lower-row caged animals tend to "escape" into transfer boxes readily, while upper-row caged animals often stubbornly resist leaving their cages and exiting into transfer boxes. In a quantitative study I did on 20 pair-housed cynos, the animals spent 94 percent of their waking time in the upper part of the vertically arranged double cage. All food was given in the bottom section, yet the animals would bring the food to the upper part and consume it there. The monkeys' preference along the gradient of height was unequivocal!

Your observation is similar to mine. Two pair-housed female rhesus macaques visited the top half of their double-cage significantly more often and spent significantly more time there than in the bottom half of their double-cage (Clarence

et al., 2006). MacLean and Roberts-Prior (2006) concluded from detailed studies with rhesus, that the monkeys' consistent preference for the upper-row reflects the paramount importance of access to elevated space.

I observed squirrel monkeys in vertically arranged double cages and also found that the animals clearly preferred the upper half of their cages. The only time they went to the bottom half was when they retrieved a toy or picked through the bedding for treats.

When visiting facilities that have their pair-housed macaques in vertically arranged double-cages, I repeatedly got the impression that subordinate partners are disadvantaged in this caging system, with dominant animals preventing subordinates from spending as much time in the upper section as they would like to.

Your impression is right in many situations. I see this happening often with our pair-housed cynos. Most of our pairs get along great, and both partners usually sit together in the top section of the double cage. Some pairs, however, do not get along so well, and one monk is at the bottom of the cage most of the time looking very worried, while the other monk spends almost all the time in the top part of the cage. If this situation goes on for a few days, we separate the animals and match them up with other more compatible companions.

Salzen (1989) observed small groups of squirrel monkeys in vertically interconnected cage units. The animals showed a preference for the upper cages, and subordinate females were liable to stay in the lower cages. Obviously, the animals competed over access to the preferred upper cages, with subordinate animals obviously being disadvantaged.

Does anyone work in a facility that has successfully dealt with the illumination differences in cages arranged in multi-tier racks?

I do not think the researchers over here have given it any thought. I do not even think that it crosses their minds that the quantity and/or quality of light their mice are receiving could affect the findings of their studies with these animals.

The differences in illumination in upper- versus lower-tier cages are indisputable (Figure 64). Clough (1982) is probably not exaggerating when he states that light intensity in the cages is likely to be the most variable environmental factor in the average animal room.

The Animal Welfare Regulations of the US Animal Welfare Act admonish that:

> *Lighting must be <u>uniformly</u>* [emphasis added] *diffused throughout animal facilities and provide sufficient illumination to aid in maintaining good housekeeping practices, adequate cleaning, adequate inspection of animals, and for the well-being of animals* (US Department of Agriculture, 1991).

Evan MacLean

Figure 64

In the standard double-tier caging system for macaques animals in the top row live in a quasi-arboreal bright environment, while animals in the bottom row live in a cave-like environment to which they are not biologically adapted.

This legal mandate cannot be met when animals are kept in the traditional multi-tier caging systems: While animals in the upper row live in well-illuminated quarters those in lower rows often live in a semi-gloomy environment often making it necessary for care personnel to use flashlights in order to identify individual animals and assure the adequate cleaning of the cage (Figure 64; Reasinger and Rogers, 2001).

The National Research Council (1996) advocates rotating cage position relative to the light source to account for the different housing environments of animals kept in upper-row versus lower-row cages. I very much question if this is an acceptable "trick" or if it simply "rotates" the problem without fixing it. If anything, rotating cage position is likely to make the methodological situation even worse, by introducing another source of variance.

The differences in light could be addressed for rodents, by providing all animals with a species-appropriate shelter or nest. The animals will hide and rest in these dark places most of the time, thus being exposed to much more equal illumination.

In order to bring more light into the lower-row cages of macaques, I had all solid side panels replaced with mesh walls, allowing more light reflecting

into the cages. This modification more than doubled the light intensity in the lower-row cages (Reinhardt et al., 1992), but it did not eliminate the significant illumination difference between upper- and lower-row cages.

The "tier effect" is a variable that does not necessarily invalidate research findings, but it *must* be accounted for in the statistical analysis. Of course this does not address the welfare issue, which should also be carefully considered for each species.

9.6. Individually Ventilated Caging (IVC)

Those of you who have first-hand experience with both the individually ventilated caging system and the traditional caging system, which system is more animal welfare conducive? Economical factors should not influence your decision, please, only the standpoint of the caged animal.

I believe that in terms of animal welfare, the development and uptake of individual ventilated cage systems is one of the worst "advances" in laboratory animal housing. I am regularly told that environmental enrichment is a threat to biomedical research and that more studies have to be conducted before enrichment can be adopted by the research industry, but so many labs have gone over to one of the many, extremely various ventilated systems without raising this same objection. The lack of data in this subject boggles the mind, given the numerous variables that come along with this caging system, for example, sound attenuation, smell attenuation, sensory deprivation, vibration, ultrasound, reduced handling, and movement to less preferred bedding types.

In our lab, IVCs are used only when researchers need frequent access to animals who are immune compromised and, consequently, would not survive in open top cages, e.g., SCIDs (severe combined immunodeficieny disorder), nudes and several strains of knockout mice.

We have not observed any detrimental effects of this caging system on the mice. However, IVCs and the associated equipment are relatively expensive and very labor intensive, so they are not something we would choose on purely economic grounds. There are two other drawbacks: The contact between animals and care personnel is reduced, and the complicated technology creates a comparatively high risk that something goes wrong. My biggest fear is a power outage, where the emergency back up does not kick in and whoever is on duty does not realize the implications and forgets to phone me.

We have better success with trio-housing male mice in IVCs than in open bins. During a follow-up period of four months no fights were reported among males kept in the IVCs, while in a parallel study of trio-housed mice in open bins, six groups had to be split up due to fighting.

We, too, have more luck with group-housing males in IVCs than in the static cages. In addition, we have better reproduction rates in breeding colonies kept in IVCs, which I believe is due to the relatively infrequent handling of the female and her litter, plus the longer period of time that they can stay in one and the same nest.

Some strains will build their nest very close to the air valve, while others build it as far away from it as possible. The mice build elaborate nests over the two weeks between cage changing. We try not to discard the whole nest but move parts of it that are dry into the new cage, which will then already have the familiar scent of the old nest. As for environmental enrichment, we have found a way of providing shelters without interfering with the ventilation too much, by using pipette boxes that have been cut in half. We place these in the front of the cage, with the opening facing into the cage. The mice use these shelters for sleeping and nesting. The great thing about these is that the labs provide them to us for free, the carpenter shop cuts them for a nominal fee, and they are autoclavable and disposable.

If properly adjusted to the animals' behavioral needs, such as building nests and sleeping in a nest or shelter, and if properly and reliably serviced, an individually ventilated caging system can enhance animal welfare.

9.7. Restraint Tubes for Rodents

Rodents are often restrained for blood collection and injection, by coaxing the animals into little tubes. I wonder if this kind of enforced immobilization is not introducing stress as an uncontrolled variable into the data collected from such animals?

We had a group of visiting scientists who used tube-restraint as classical stressor for experimental purposes in their research facility. When they saw us working with our rats, they could not believe their eyes: Our rats were quite happy to crawl into the tubes, go to sleep and show no apparent signs that they had become stressed by the procedure. It may well be that our rats were particularly good-natured and laid back and/or were so well habituated to being 30-minute tube-restrained, that they calmly accepted the situation.

I also find that rats, mice and guinea pigs will enter restraint tubes quite happily, provided I am patient and gentle-and-firm the first couple of times when I prompt them to crawl into a tube. The initial experience associated with the tube is probably the determining factor in the restrained subject's response to subsequent restraint sessions. I encourage our researchers to handle their animals daily during the week *prior* to the actual studies. On these occasions they will also tube-restrain their animals without doing any other procedure that could possibly cause pain to the animals. This preliminary routine assures that the subjects not only will be familiar with the researcher, but that they also will be well acclimatized to the tube at the beginning of the study. I think this provides a good condition for the animals to

experience little or no restraint-related stress during the experiment.

Either we use ordinary transparent restraining tubes that we cover with a paper towel, or we use opaque tubes made of red Perspex so that they become a dark, "safe" hiding place. Since the scent of a stranger adhering to the tube is likely to induce a negative reaction in rodents, we thoroughly rinse the tubes between cages. We did notice that the animals are more reluctant to crawl into the tube and tend to be restless in the tube when we skipped the rinsing. With a bit of "training" rodents do enter such tubes without appearing to be stressed, and as already pointed out, they will often fall asleep after a few minutes. They do give the impression of being relaxed, even though we take tail-cuff blood pressure readings, or withdraw blood from previously implanted cannulae at various time points. When I take the animals out of the tube after a procedure, they are not agitated and usually resume their routine business, such as exploring the environment and grooming themselves. I really believe that gently habituated rodents do not experience undue stress, or any stress at all, while they are restrained in dark tubes during noninvasive procedures. I should perhaps emphasize, it is very important to make sure that the animals do not get overheated while they are restrained in the tubes. They can get hyperthermic very easily, and this will certainly distress them; they will come out of the tubes in a state that I can only describe as "prostrated"—reluctant to move, panting, semi-conscious, damp or moist. This *must* be avoided, and it can be avoided by keeping the animals in the restrainer for only short periods at a time.

Would you recommend to always keep the restraint tube-environment dark, or has your experience shown that it does not make a noticeable difference whether a rodent is restrained in a transparent tube or a dark tube?

We use the typical transparent plastic tube, which I always cover with a surgical drape to darken it, so that the animals feel relatively secure. After all, their natural instinct is to seek a dark shelter in the event of danger, and being coaxed into a tube by a human hand must, indeed, be rather scary for them.

It is my experience that, if the tubes are red Perspex or covered with paper or surgical drape, the rodents seem to be relaxed and remain relaxed throughout the procedure, even if I draw a blood sample—which, I guess, must cause some discomfort despite the use of topical analgesics. When the tubes are transparent and uncovered, the animals will often wriggle about in what I presume is an attempt to get out—our tubes open at both ends, so the animals do not have to come out backwards unless they want to.

Enforced restraint is not an intrinsic stressor for rodents. If an animal has been well familiarized with the handling personnel and with the restraint tube, the tube kept dark and the duration of the restraint session short enough to forestall overheating, behavioral signs of stress can be avoided. It needs to be demonstrated whether physiological stress parameters reflect baseline values in animals who seem to accept tube-restraint.

10. Miscellaneous

10.1. Marking Mice for Video Recording

We plan to video-record groups of black mice and would like to somehow identify individuals in the recordings. Can anybody share experiences on how to mark rodents for individual identification?

I use a human hair bleach to individually mark my dark C57Bl/6J mice. Contrary to what might be expected, this shows up very well under infra-red, under some circumstances even better than under white light. Because mouse hair grows so quickly, you will have to re-apply the marks every few weeks. Some mice do develop bald patches at the site of bleaching. We believe this is due to the hair follicle becoming slightly damaged and the hair falling out as a consequence of normal grooming. I do not consider this as a welfare problem, as the skin is not reddened, hence probably not inflamed.

I do not know if they will show up under infra-red, but we always use histological dyes—neutral red, malachite green and crystal violet made up as a concentrated solution in 70 percent alcohol—to identify mice. Applied with a cotton bud, these marks last at least a week.

What an excellent idea!

There are several possibilities for marking dark mice. The application of histological dyes seems to be a perfect option.

10.2. Mice Who Do Not Reproduce

We have been trying to breed wild forest mice for almost a year now, and *no* litters have been born. The animals are pair-housed in standard cages; they do have nesting material and shelters. Any suggestions as to what we can do to get these animals to reproduce?

Wild mice usually breed in lab conditions reasonably well. I would suggest to breed your mice in larger than standard-size cages. As a general rule, wild mice breed

better the larger their cage. Trios—two females and one male—tend to reproduce better than pairs, probably because a wild female mouse likes to share a nest with another female. They need more nesting and bedding material than usual, as they like to bury their young. Try not to let them get too fat, and breed as young as possible. The breeding success can be enhanced when you try adjusting daylight length and light intensity to mimic a biologically natural light cycle; this will fool your mice—just as ours—into believing that it's time to mate.

At our facility, we keep colonies of wild mice in 2 m x 3 m large enclosures with 100 cm high metal walls. The floor is wood and covered with a layer of shavings and/or hay. The enclosure is provisioned with cardboard boxes, bricks and other objects behind which and in which the mice can hide. Overcrowding can quickly become a problem!

I am impressed and very pleased. It took only 32 minutes to receive your really good advice. Thank you!

Wild mice do reproduce very well when the housing and living conditions are mice-appropriate.

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12. Subject Index

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